PROGRAM MANAGER FOR
ROCKY MOUNTAIN ARSENAL

U.S. ARMY
MATERIEL COMMAND

— COMMITTED TO PROTECTION OF THE ENVIRONMENT —

# FINAL
# SUPPLEMENTAL FIELD STUDY
# PHASE I PLAN

AUGUST 1994

NTRACT NO. DAAA05-92-D-0002, Delivery Order 0004

**EBASCO SERVICES INCORPORATED**
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TECHNICAL SUPPORT FOR
ROCKY MOUNTAIN ARSENAL

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# SUPPLEMENTAL FIELD STUDY
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Prepared by:

EBASCO SERVICES INCORPORATED

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THE OBJECTIVE OF THE RMA SUPPLEMENTAL FIELD STUDY (SFS) IS TO RESOLVE THE BIOMAGNIFICATION FACTOR (BMF) DISPUTE ISSUE THAT WAS RAISED BY THE EPA UPON ITS REVIEW OF THE AUGUST 1993 DRAFT FINAL INTEGRATED ENDANGERMENT ASSESSMENT/RISK CHARACTERIZATION (IEA/RC) REPORT. THE PROGRAM INVOLVES TWO PHASES. PHASE I WILL INVOLVE COLLECTING AND ANALYZING BIOTA TISSUE SAMPLES FROM THE RMA "AREA OF DISPUTE" (AOD), A SPECIFIC AREA OVER WHICH THE ECOLOGICAL RISK CHARACTERIZATION IN THE AUGUST 1993 DRAFT FINAL IEA/RC IS CONTESTED. IF PHASE I INDICATES THAT UNACCEPTABLE RISKS TO BIOTA ARE LIKELY, THEN THE SUPPLEMENTAL STUDY MAY PROCEED WITH PHASE II TO COLLECT ADDITIONAL TISSUE AND SOIL DATA TO ESTIMATE BMFS FOR SELECTED SPECIES. THIS REPORT IS ORGANIZED INTO THE FOLLOWING SECTIONS AND APPENDICIES: SECTION 1.0- INTRODUCTION. SECTION 2.0- PHASE I DESIGN OBJECTIVES AND CONDITIONS. SECTION 3.0- STUDY DESIGN. SECTION 4.0 REFERENCES. APPENDIX A- BIOTA FIELD SAMPLING PROCEDURES. APPENDIX B- FURTHER STATISTICAL DISCUSSION AND EVALUATIONS.

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| | |
|---|---|
| AOD | Area of Dispute |
| BCRL | Below Certified Reporting Limits |
| BEMA | Bald Eagle Management Area |
| BFDF | Biota Sample Field Data Form |
| BMF | Biomagnification Factor |
| C-O-C | Chain-of-Custody |
| CMP | Comprehensive Monitoring Program |
| CRL | Certified Reporting Level |
| CV | Critical Value |
| DDE | Dichlorodiphenylethene |
| DDT | Dichlorodiphenyltrichloroethane |
| DQO | Data Quality Objectives |
| E | Error Term |
| EA | Endangerment Assessment |
| EPA | U.S. Environmental Protection Agency |
| FDF | Field Data Form |
| FFDF | Fortuitous Sample Field Data Form |
| FR | Prey Fraction |
| ft | Foot/Feet |
| g | Gram |
| GC/ECD | Gas Chromatography/Electron Capture Detection |
| GI | Gastrointestinal |
| GIS | Geographical Information System |
| GLP | Good Laboratory Practices |
| GPS | Geographical Positioning System |
| HA | Alternative Hypothesis |
| HASP | Health and Safety Plan |
| HI | Hazard Index |
| H0 | Null Hypothesis |
| HQ | Hazard Quotient |
| i | Sampled Species |
| ID | Identification |
| IEA/RC | Integrated Endangerment Assessment/Risk Characterization |
| IRDMIS | Installation Restoration Data Management Information System |
| j | Predator Species |
| LECA | Black-Tailed Jackrabbit, *Lepus californicus* |
| LT | Less Than |
| m | Meter |
| MATC | Maximum Allowable Tissue Concentration |
| MDL | Method Detection Limit |
| MRL | Method Reporting Limit |

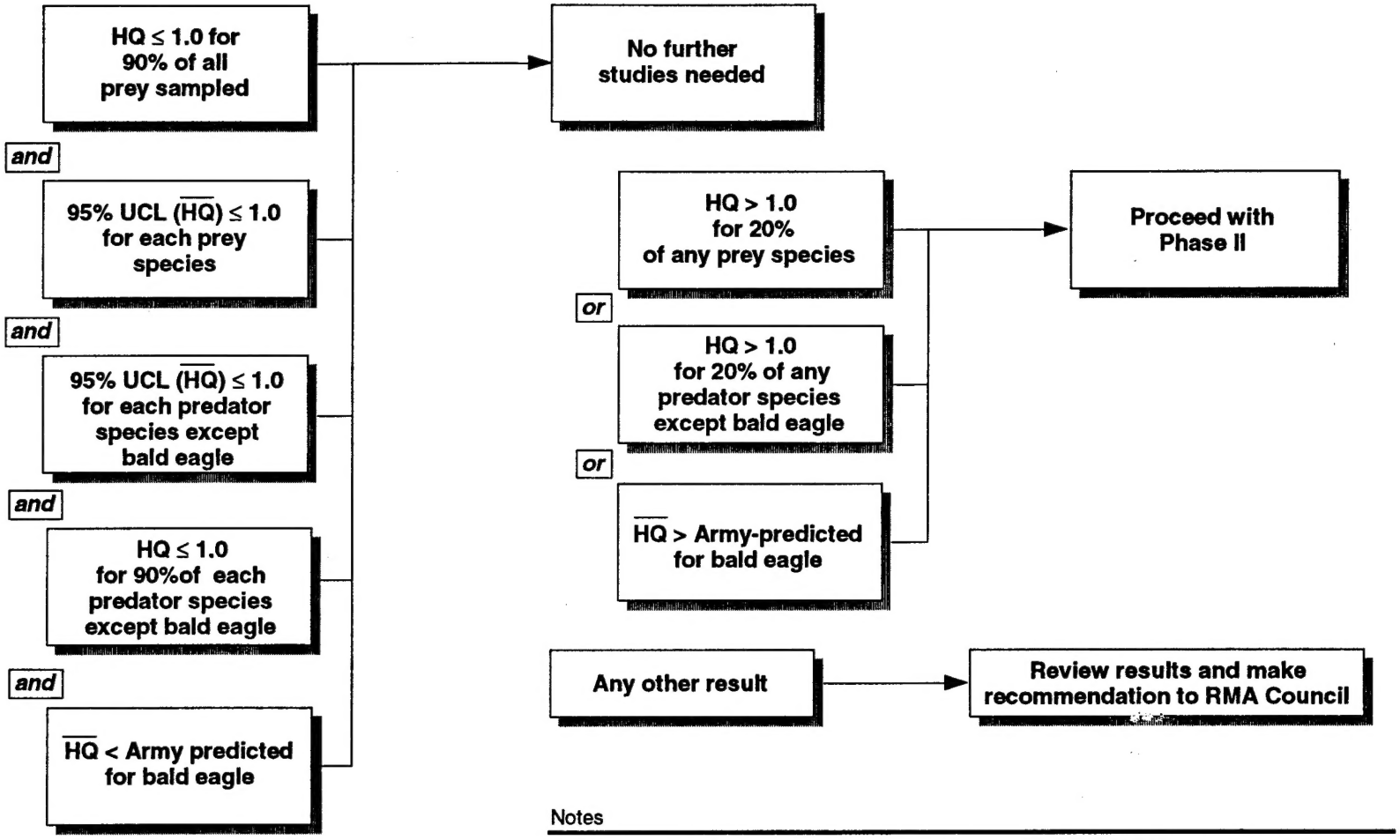
| | |
|---|---|
| n | Sample Size |
| NCMIS | Negative Chemical Ionization Mass Spectrometry |
| OCP | Organochlorine Pesticide |
| OS | Order Statistic |
| QA | Quality Assurance |
| QC | Quality Control |
| R | Feeding Rate |
| RMA | Rocky Mountain Arsenal |
| SAP | Sampling and Analysis Plan |
| SD | Standard Deviation |
| SFS | Supplemental Field Study |
| Shell | Shell Oil Company |
| SIM | Selected Ion Monitoring |
| STP | State Planar |
| STVU | Starling, *Sturnus vulgaris* |
| TC | Tissue Concentration |
| TRL | Target Reporting Limit |
| TRV | Toxicity Reference Value |
| TSP | Trisodium Phosphate |
| UCL | Upper Confidence Level |
| USAEC | U.S. Army Environmental Center |
| USFWS | U.S. Fish and Wildlife Service |
| USGS | U.S. Geological Survey |

## 1.0 INTRODUCTION

The objective of the Rocky Mountain Arsenal (RMA) Supplemental Field Study (SFS) is to resolve the biomagnification factor (BMF) dispute issue that was raised by the Environmental Protection Agency (EPA) upon its review of the Draft Final Integrated Endangerment Assessment/Risk Characterization (IEA/RC) Report (EBASCO 1993a). The program involves two phases (SFS-Phase I and SFS-Phase II). SFS-Phase I will involve collecting and analyzing biota tissue samples from the RMA "area of dispute" (AOD), a specific area over which the ecological risk characterization in the August 1993 Draft Final IEA/RC is contested as defined by the RMA Council (see Section 2.4). Additional sampling of selected species will also be conducted in the part of the eagle exposure area that is in the Bald Eagle Management Area (BEMA) or April 1993 prairie dog towns, but outside the AOD. If SFS-Phase I work indicates that unacceptable risks to biota are likely, the supplemental study may proceed with Phase II to collect additional tissue and soil data to estimate field BMFs for selected species.

The specific objective of the SFS-Phase I is to determine whether any of a set of risk-based criteria for proceeding to a SFS-Phase II sampling program are exceeded. These criteria are presented in Figure 1, which is discussed in more detail in Section 2. The seven-step data quality objective (DQO) process discussed in the EPA's Guidance for Planning for Data Collection in Support of Environmental Decision Making Using the Data Quality Objective Process (1993) provides the framework for this SFS-Phase I design. Steps 1 through 6 are described in Sections 2.1 through 2.6, respectively, and Step 7 is described in Section 3.

A recommendation based on the results of the SFS-Phase I analyses will be provided to the RMA Council, which will determine whether the SFS-Phase II will be implemented. In addition, the SFS-Phase I is expected to provide new information on the magnitude and extent of potential excess risks to biota in the AOD and on the predictive capabilities of the existing IEA/RC ecological risk estimation methods, although it is not designed for these purposes.




Notes

For predator species, hazard quotients are ratios of doses to TRVs.

For prey species, hazard quotients are ratios of tissue concentrations to MATCs.

If Phase II is not triggered by analyzing HQ statistics, then HIs will be calculated for predators based on the sum of the HQs for aldrin/dieldrin, DDE/DDT, and endrin.

RMA SFS - Phase I Plan 8.94.jb

**Figure 1**

**Decision Process for SFS - Phase I**

Rocky Mountain Arsenal
Prepared by: Ebasco Services Incorporated

## 2.0 SFS-PHASE I DESIGN OBJECTIVES AND CONDITIONS

### 2.1 DQO STEP 1—PROBLEM STATEMENT

Step 1 involves concisely defining the problem that requires new environmental data and identifying planning team members and the decision maker (EPA 1993). The problem to be solved by the SFS-Phase I is to determine whether a collocated soil and tissue sampling program (i.e., SFS-Phase II) should be conducted over the AOD to resolve the EPA BMF dispute issue (i.e., is there sufficient risk to the biota in the AOD to warrant additional studies). The SFS-Phase II sampling program would be designed to reduce the reliance on disputed statistical methods and assumptions used to derive BMF estimates from the currently available RMA tissue and soil concentration database.

The planning team for the SFS-Phase I will consist of members of the RMA Endangerment Assessment (EA) Technical Subcommittee. There will be no primary decision maker; all decisions will be made by consensus.

### 2.2 DQO STEP 2—DECISIONS TO BE MADE

Step 2 involves defining the decision that will be made using the data to be collected in the sampling program. The outputs from this step are a statement of the decision, and a list of actions or possible outcomes that would result from each resolution of the decision (EPA 1993).

The decision that will be resolved with the SFS-Phase I data (the "Phase I decision") is whether the potential risks to prey or predators (estimated from SFS-Phase I tissue concentration data) are high enough to merit a collocated soil and tissue sampling program (i.e., SFS-Phase II) for the RMA area of dispute. The decision process is further described in Section 2.5 and Figure 1.

The actions that would result from an affirmative response to the SFS-Phase I decision are the following:

- A recommendation to the RMA Council to perform a SFS-Phase II sampling program.

The action that would result from a negative response is the following:

- A recommendation to the RMA Council that the SFS-Phase I results do not justify a SFS-Phase II sampling program and further definition of any areas of risk if they are identified.

## 2.3 DQO STEP 3—INPUTS TO THE DECISION

Step 3 involves identifying the criteria that will be used to determine the need for the SFS-Phase II and to determine which variables require environmental measurement (EPA 1993). The criteria that will be used include the following:

- A maximum allowable tissue concentration (MATC) for each of the species sampled in the Phase I design for which potential risk is to be evaluated
- A toxicity reference value (TRV) for each species that is not sampled in the SFS-Phase I design (i.e., the top predators), but for which potential risk is to be evaluated
- A prey fraction ($FR_{i,j}$) for each of the sampled species (i) that will be used as a component of the dose to a predator species (j) for which potential risk will be estimated
- A feeding rate ($R_j$) for each predator species for which potential risk will be evaluated
- Measurements of tissue concentrations of each chemical for each of the sampled prey species (for which potential risk will be evaluated and that will be used as a component of the dose to a predator species for which potential risk will be estimated)
- The number of usable samples (n) collected for each of the measured environmental variables

The environmental variables that will be measured include the tissue samples that will be collected and analyzed as described in Sections 3.3 and 3.4.

## 2.4 DQO STEP 4—BOUNDARIES OF THE STUDY

Step 4 involves providing a description of study-area characteristics, including a detailed description of the spatial and temporal boundaries of the study area and a description of any constraints that may interfere with the study (EPA 1993).

RMA Council guidance specified that a general AOD be defined based on disagreement among the three hazard quotient (HQ) estimates (based on Army, EPA, and Shell BMF approaches) regarding exceedance of the action level of 1.0. Although the area of disagreement over potential HQ exceedances varies substantially by trophic box and chemical, the RMA Council defined the AOD based on predicted aldrin/dieldrin HQs for small mammals. This AOD will be used to estimate HQs for prey based on concentrations in their tissues and HQs for all predators (except the bald eagle) based on the dose they receive in the AOD.

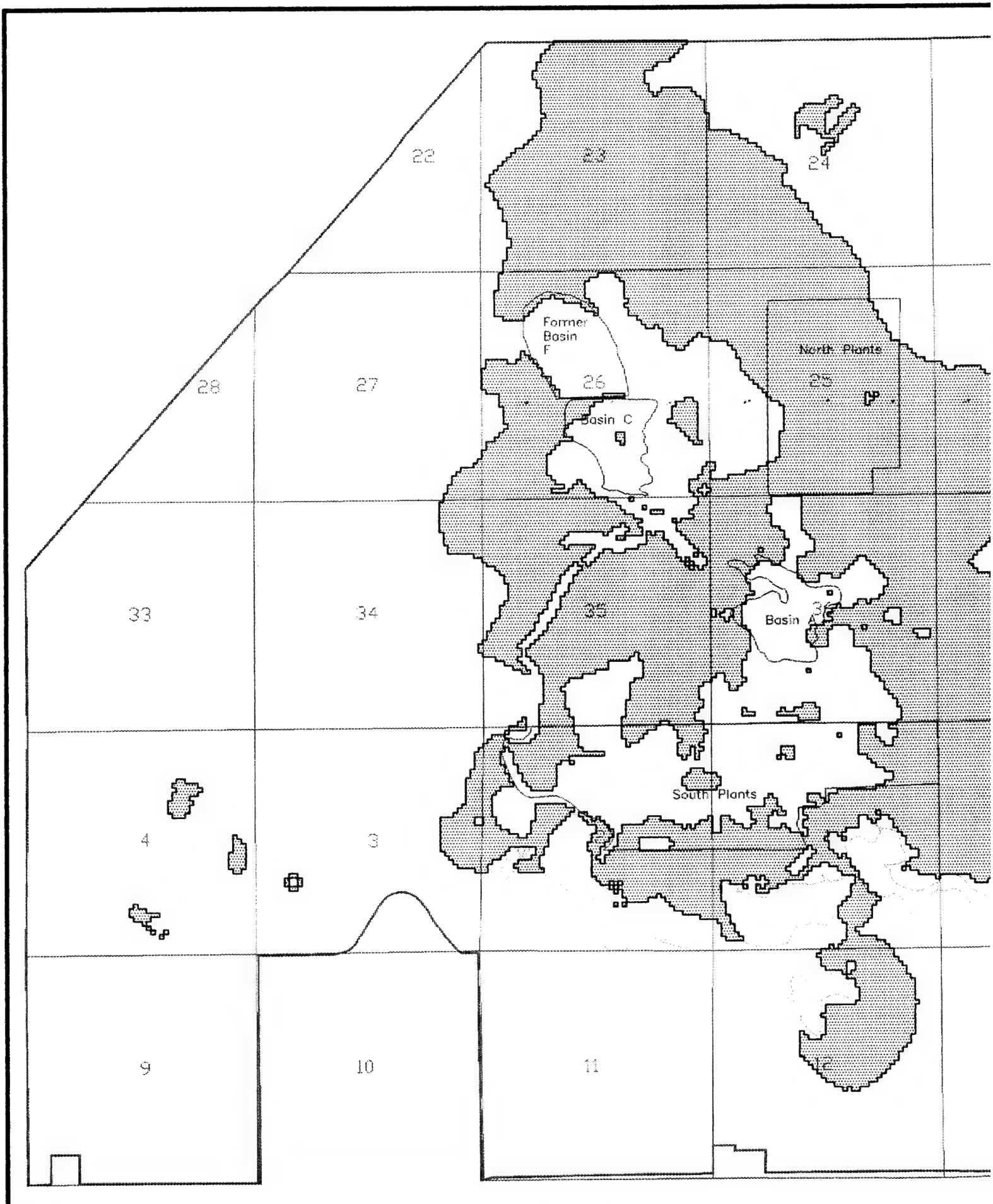
The study area for the SFS-Phase I is shown in Figure 2. The inner boundary of the AOD is the perimeter of the area in which all three aldrin/dieldrin HQs are greater than 1.0. The outer boundary is the perimeter of the area in which the EPA aldrin/dieldrin HQ is greater than 1.0. In defining the AOD, the HQs are calculated as the ratio of predicted tissue concentration (TC) to MATC.

For the bald eagle, the exposure area for a single individual was defined in the Proposed Final IEA/RC (EBASCO 1994a) as the BEMA and all prairie dog towns that were outside the BEMA as of April 1993. This area is in exceedance for the eagle according to the Army and EPA HQ predictions, but not according to the Shell HQ prediction. To better evaluate the risk to individual eagles, additional sampling of prairie dogs will be conducted in that part of the eagle exposure area that falls outside the AOD (Figure 3).

SFS-Phase I sample collection is proposed for the field season of 1994 (May through September). Sampling times for individual species are related to their life cycles, as described in Section 3.3, 3.4, and Appendix A. The results of the study must be available as soon as possible after evaluation, including proper quality control (QC), i.e., approximately 3 to 6 months after the last samples are collected.

## 2.5 DQO STEP 5—DECISION PROCESS

Step 5 involves integrating the products of Steps 1 through 4 into a single statement that describes the logical basis for choosing among alternative actions (i.e., recommending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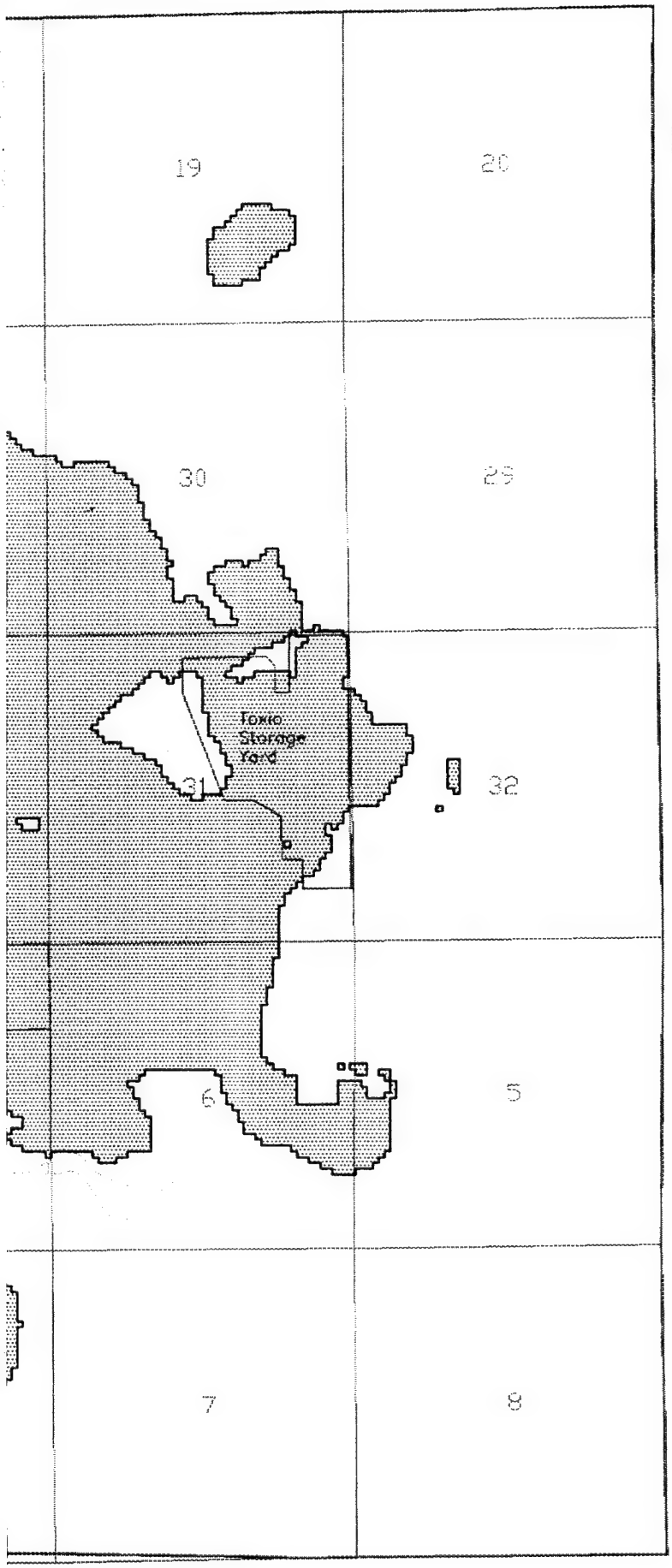


## Legend

Area of Dispute and Prey Study Area

Lake

31 Section Number

Section Line

N

0 2000 4000

Scale in Feet

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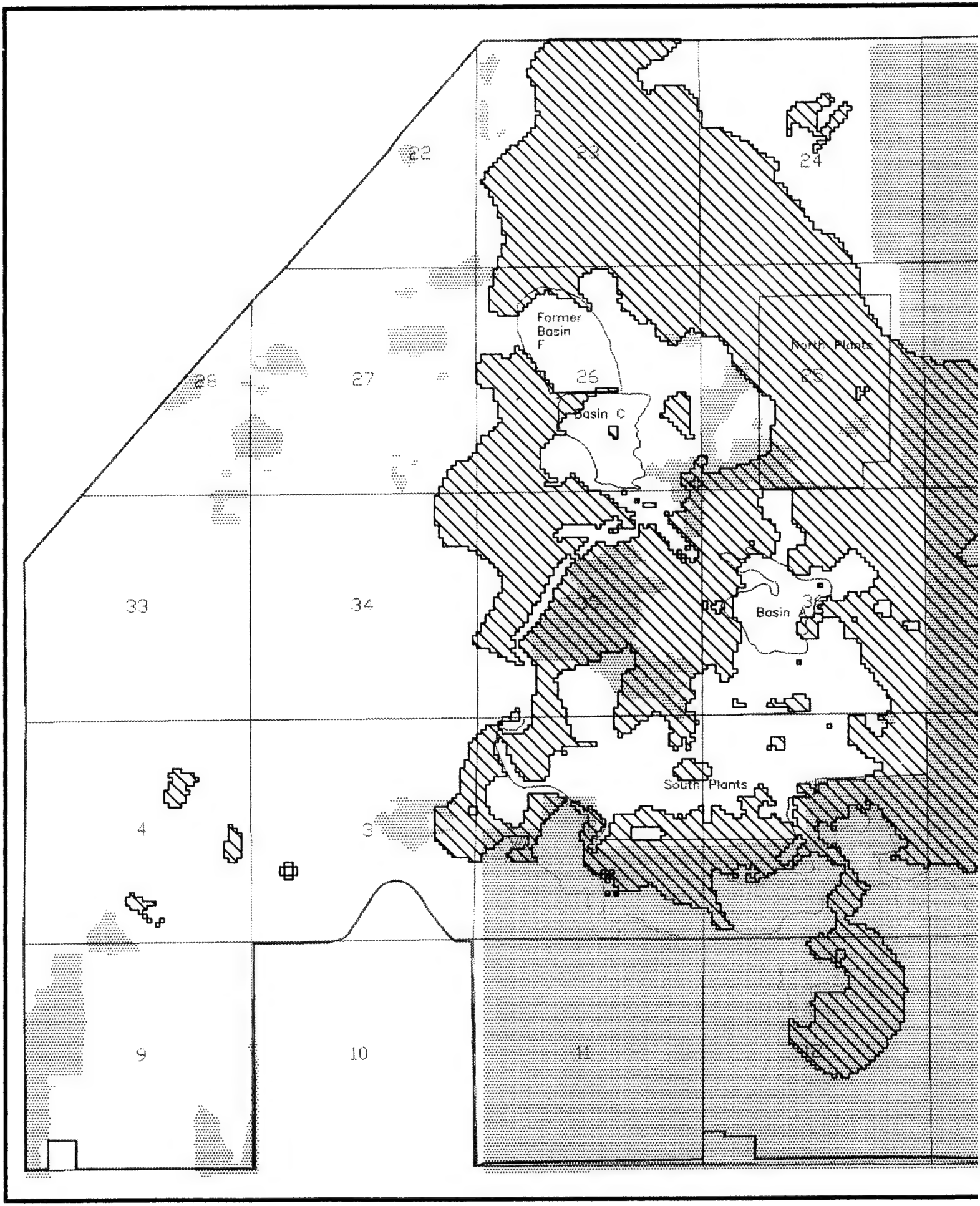
for Rocky Mountain Arsenal

May 1994

Figure 2

Supplemental Field Study Phase I
Area of Dispute

Rocky Mountain Arsenal
Prepared by: ENSERCH Environmental Corp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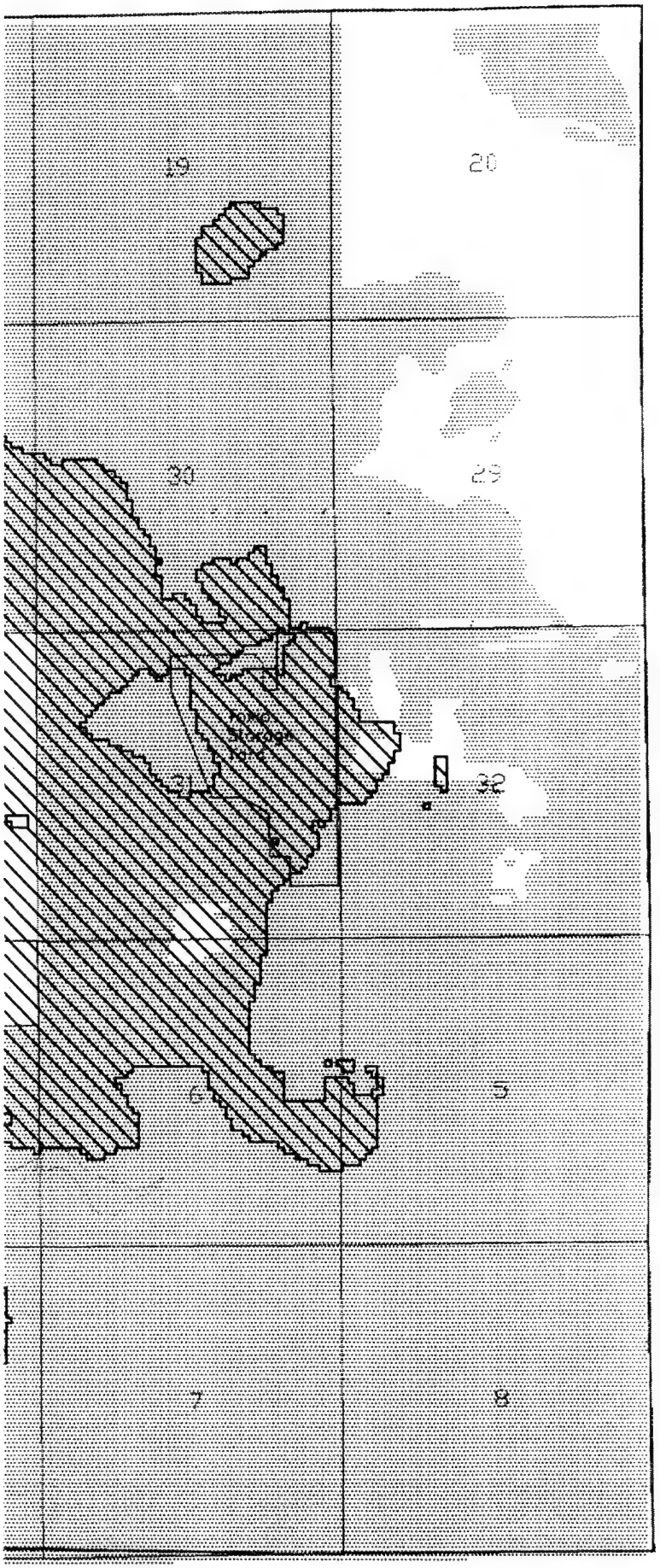


## Legend

- Area of dispute
- Bald eagle exposure area inside the area of dispute
- Bald eagle exposure area outside the area of dispute

Lake

31 Section Number

Section Line

-N-

0 2000 4000

Scale in Feet

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Figure 3

Supplemental Field Study Phase I
Bald Eagle Exposure Area Outside the
Area of Dispute

Rocky Mountain Arsenal
Prepared by: ENSERCH Environmental Corp

SFS-Phase II, not recommending SFS-Phase II, or performing additional analyses of SFS-Phase I data before making a recommendation). The output from Step 5 is to be the "if...then..." statement that defines the conditions that would allow the decision maker to choose one of these three alternatives (EPA 1993).

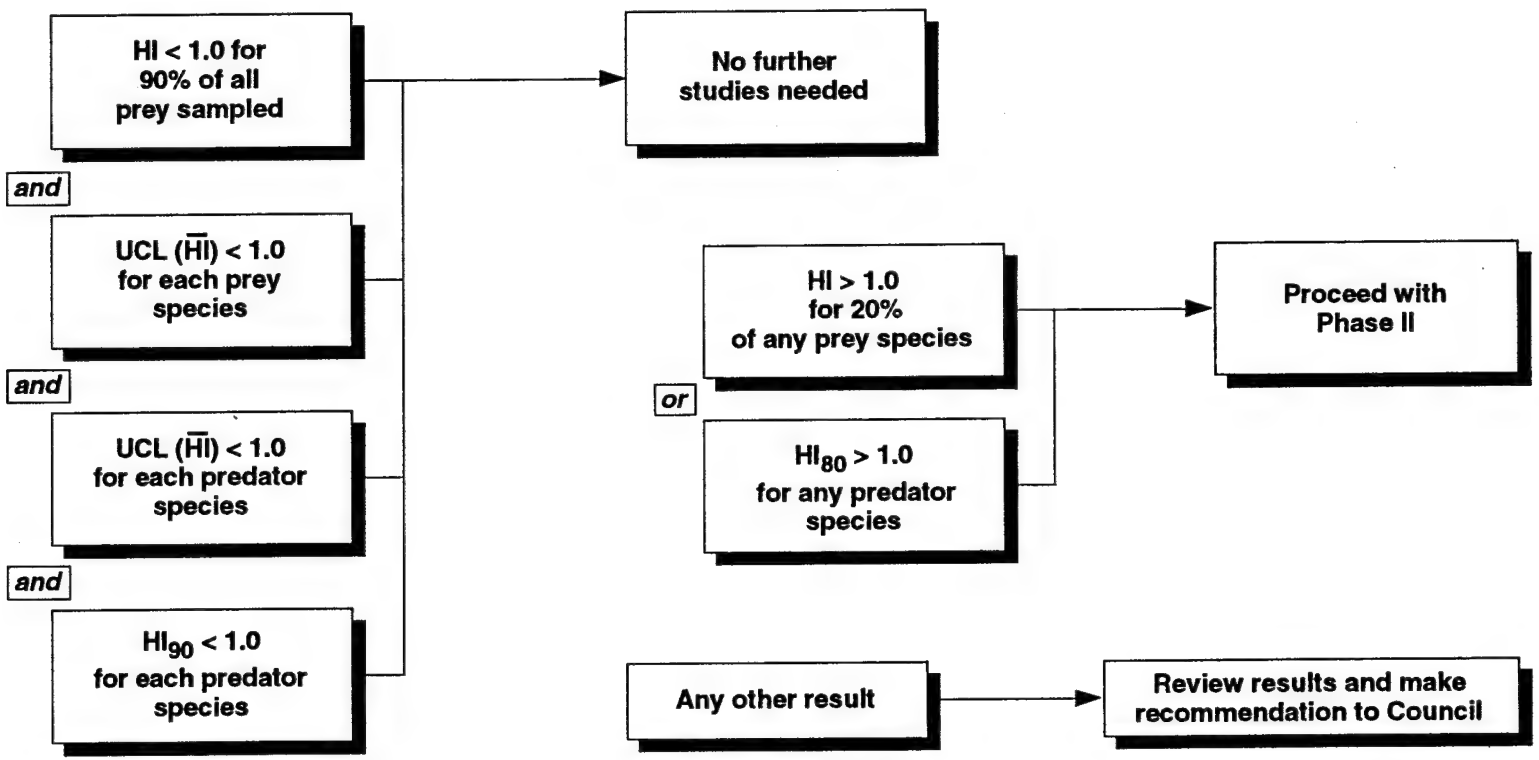
A decision process (Figure 4) for the SFS-Phase I was initially proposed by the RMA EA Technical Subcommittee in its conceptual design document Rocky Mountain Arsenal Supplemental Field Study (EBASCO 1993b). This initial decision process was accepted by the RMA Council; however, on the basis of technical discussions following the RMA Council's decision, the following two modifications were made to the decision process. First, hazard indices (HIs) were changed to aldrin/dieldrin HQs to be consistent with the delineation of the AOD. This does not preclude the estimation of HQs for the remaining organochlorine pesticides (OCPs). Second, bald eagles were separated from the other predators because the exposure assessment procedure used in the IEA/RC averages the exposure of the bald eagle for a single exposure range within RMA (i.e., the BEMA plus all prairie dog towns present in April 1993 and outside the BEMA). Therefore, a single mean HQ value (meanHQ) (no percentiles) will be estimated.

The revised decision process that will serve as the basis for the SFS-Phase I sampling design is presented in Figure 1.

## 2.6 DQO STEP 6—ACCEPTABLE LIMITS ON DECISION ERROR

Step 6 involves specifying the decision makers' acceptable limits on decision errors, which are used to establish appropriate performance goals for limiting uncertainty in the data. The output from Step 6 is to be the decision makers' (i.e., RMA EA Technical Subcommittee's) acceptable decision error rates based on a consideration of the consequences of making an incorrect decision (EPA 1993).

The decision process proposed by the RMA EA Technical Subcommittee as revised (Figure 1) partially specifies its decision-error tolerances through the use of confidence limits on meanHQs




RMA SFS-Phase I Plan 8.94.jb

**Figure 4**

**Decision Process for SFS - Phase I Risk Criteria**

Rocky Mountain Arsenal
Prepared by: Ebasco Services Incorporated

and percentiles on individual HQs. The calculations presented in spreadsheets contained in Appendix B describe how to determine the width of these confidence intervals modified to HQs as a function of number of samples collected and assumed variance. The spreadsheets also describe how to determine sample size as a function of the desired statistical power, confidence level, and error tolerance. For example, they describe how to select the sample size that will allow a difference of 0.2 (the error tolerance) between the true meanHQ and the action level (meanHQ = 1.0) with 95 percent confidence (the confidence level) to be detected 80 percent of the time (the power of the statistical test). The confidence limits account for potential error in estimating the mean due to a variety of sources (e.g., random sampling error, analytical error).

The analysis of these statistical components of the decision error performed to date provides a means for evaluating either the required number of samples to achieve a specified acceptable-error tolerance or the error level associated with a specified number of samples. Hard copies of the spreadsheets developed to perform the necessary statistical calculations are provided in Appendix B.

## 3.0 DQO STEP 7—STUDY DESIGN

Step 7 involves identifying the most resource-effective sampling and analysis design for generating data that are expected to satisfy the DQOs. This design is to be selected from a group of alternative designs generated during this step (EPA 1993).

The design described below was selected from a statistical and practical evaluation of several alternatives and is based on the specific study objectives, conditions, and decision process defined in Section 2 and Figure 1. The formal basis for the decision, species to be sampled, sample collection, sampling type, sampling locations, number of samples, and laboratory analysis of samples are described in Sections 3.1 through 3.8. A summary of recommendations is provided in Section 3.7. Details of the statistical design methods, calculations, and rationale are provided in Appendix B.

### 3.1 BASIS FOR DECISION

The basis for decision, as described in Figure 1, includes the comparison of the following statistics to an action level of 1.0: prey and predator upper 95 percent confidence limit on the mean aldrin/dieldrin HQ (meanUCL), and predator and prey 90th percentile aldrin/dieldrin HQ (HQ90). In addition, the estimated mean HQ for eagle is compared to that predicted based on the Army BMFs for prey. The decision process shown in Figure 1 implies a set of hypothesis tests that give direct information regarding the statistical adequacy of the sample size and other aspects of the study design. The decision boxes in Figure 1 that pertain to the 95 percent or 80 percent UCL (meanHQ) imply a formal statistical hypothesis (i.e., meanHQ is to be tested at the 95 percent or 80 percent confidence level). In contrast, the decision boxes pertaining to percentiles of HQ or the estimated meanHQ (not the UCL) do not imply a formal statistical hypothesis test because confidence is not specified. The formal and informal tests implied by Figure 1 are given below; each is comprised of a null hypothesis (H0) and an alternative hypothesis (HA). Because the tests pertaining to the 20th percentile (i.e., the center boxes in Figure 1) are less stringent than those pertaining to the 95 percent confidence level or 90th percentile, the 80th percentile tests do not drive the selection of sample sizes and therefore are not the focus of this section.

(1) H0: prey HQ90 > 1 (i.e., HQ < 1.0 for less than 90 percent of sampled individuals for all prey species. NOTE: this cannot be statistically tested for composited species).

HA: prey HQ90 ≤ 1

and

(2) H0: prey meanUCL > 1 (i.e., meanHQ > 1 with confidence level of 95 percent for all prey species).

HA: prey meanUCL ≤ 1

and

(3) H0: predator meanUCL > 1 (i.e., meanHQ > 1 with confidence level of 95 percent for all predator species, excluding bald eagle).

HA: predator meanUCL ≤ 1

and

(4) H0: predator HQ90 > 1 (i.e., HQ < 1.0 for less than 90 percent of sampled individuals for all predator species, excluding bald eagle).

HA: predator HQ90 ≤ 1

and

(5) H0: eagle mean HQ < $HQ_{Army}$ (i.e., unbiased estimate of meanHQ is less than the HQ value predicted based on the Army BMFs for prey).

HA: eagle mean HQ > $HQ_{Army}$

The interpretation of these statements is as follows. For tests (1) and (4), H0 is rejected if, and only if, the point estimate HQ90 is less than 1.0. For test (5), H0 is rejected if, and only if, the eagle mean HQ is less than $HQ_{Army}$. For tests (2) and (3), H0 is rejected if, and only if, the UCL on the meanUCL is less than 1.0. If all five hypotheses are rejected for all species, then no further studies are needed. If not one of the three tests can be rejected for any species, then further analysis or SFS-Phase II sampling of the AOD will be considered.

## 3.2 SPECIES TO BE SAMPLED

From the list of species previously sampled at RMA under the Biota Remedial Investigation (ESE 1989), Biota Comprehensive Monitoring Program (CMP) (RLSA 1992), and the Proposed Final IEA/RC (EBASCO 1994a), species were selected during RMA EA Technical Subcommittee meetings for further sampling during the SFS-Phase I based on the following criteria:

- Total dietary fraction of prey species in diet of all predators
- Number of evaluated top-predator species or trophic boxes associated with prey species
- Categorization as a sedentary species
- Availability of a species/chemical MATC or TRV value

Information relative to these criteria is presented in Tables 1 and 2. The most important prey species (this term is used in this report to refer to species groups as well as to individual species) identified from the data in Table 1, i.e., those that are most important to the top predators being evaluated, together with their cumulative dietary fractions for the four predators with terrestrial food chains are the following: medium mammal (176.6) > insect (91.2) > small mammal (78.6) > small bird (14.5). Consideration of this information together with the other criteria presented in Table 2 resulted in the selection of prey species representing medium mammals, insects, small mammals, and small birds for tissue sampling. More specifically, the medium mammal trophic box will be represented by both black-tailed prairie dogs and rabbits (desert or eastern cottontails or black-tailed jackrabbits); the insect trophic box will be represented by grasshopper species and by beetle species; the small mammal trophic box will be represented by deer mice; and the small bird trophic box will be represented by starlings.

For the medium mammal trophic box, both black-tailed prairie dogs and rabbits were included to adequately calculate dose to both the bald eagle, which is a diurnal predator on prairie dogs, and the great horned owl, which is a nocturnal predator on rabbits. As described in Section 3.3, these six species will be collected during the appropriate season of the same collecting year.

Table 1 Summary of Ingestion/Dietary Fractions (%) by Biomass for Select Predator Species



| Prey | Eagle | Kestrel | Owl | Shorebird | Total |
|---|---|---|---|---|---|
| Soil | 2.9 | 2.9 | 2.9 | 0.0 | 8.7 |
| Plant (TR) | 0.0 | 0.0 | 0.0 | 0.7 | 0.7 |
| Earthworm | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Insect | 0.0 | 18.4 | 0.0 | 72.8 | 91.2 |
| Sm Bird | 0.3 | 12.2 | 2.0 | 0.0 | 14.5 |
| Sm Mammal | 0.0 | 66.5 | 12.1 | 0.0 | 78.6 |
| Med Mammal | 93.6 | 0.0 | 83.0 | 0.0 | 176.6 |
| Misc. Aq. | | 0.0 | 0.0 | | |
| Waterbird | 3.0 | NA | NA | 0.0 | 3.0 |
| Lg. Fish | 0.2 | NA | NA | 0.0 | 0.2 |
| Aq. Inv. | 0.0 | NA | NA | 10.5 | 10.5 |
| Sediment | 0.0 | NA | NA | 16.0 | 16.0 |
| Total | 100.0 | 100.0 | 100.0 | 100.0 | |

NA not applicable

Table 2 Criteria Information for Selection of Prey Species for SFS-Phase I Tissue Sampling



| CRITERIA: | Prey Risk | | | Predator Risk | |
|---|---|---|---|---|---|
| | Available | | Sedentary Species | Number of Predator Species | Cumulative Ingestion % |
| Prey Item | MATC[\1] | TRV[\1] | | | |
| Plant | No | No | Yes | 1 | 0.7 |
| Earthworm | No | No | Yes | 0 | 0.0 |
| Insect | No | No | Yes | 2 | 91.2 |
| Sm Bird | Yes | Yes[\2] | No | 3 | 14.5 |
| Sm Mammal | Yes[\3] | Yes | Yes | 2 | 78.6 |
| Med Mammal | Yes[\3] | Yes | No | 2 | 176.6 |

[\1] Value selected as more certain based on IEA/RC is underlined
[\2] MATC for endrin is more certain for small birds
[\3] Not available for endrin

## 3.3 SAMPLE COLLECTION

At each of the locations to be sampled, one or more individuals of the target species will be collected and prepared for chemical analysis. All collections will be coordinated through the Arsenal Activities Coordination process, with notification by Tuesday of the week prior to the planned activity, and U.S. Fish and Wildlife Service (USFWS) biologists will be included in the process to avoid conflicts with ongoing biomonitoring projects. The collected individuals will be selected to best represent animals exposed to contaminant conditions at the locations where they are collected. One-year-old nonlactating female prairie dogs or adult prairie dogs of either sex will be collected. Adult rabbits will be collected; however, it will be difficult to select a time period when young can be distinguished from adults because breeding is less synchronous than that of prairie dogs. Late instar grasshoppers will be collected, thus avoiding collection of free-flying adults. Adult ground-dwelling beetles will be collected because larvae are not readily available and ground-dwelling beetles are expected to be less mobile (and therefore have more site-specific exposure) than insects found on vegetation or free flying. Adult nonlactating deer mice will be collected. Finally, juvenile starlings will be collected, just as they are ready to fledge from nest boxes.

The seasons in which collection is expected to occur for each species are listed in Table 3. The actual time of collection must, however, be coordinated with the phenology in the year of sampling.

A variety of methods will be used to collect the specified species. Medium mammals will be collected with a trap, rifle, or shotgun, used in that order of preference and paired as collecting success or habitat differences dictate. Medium mammals that are trapped will be dispatched with carbon dioxide or a pellet gun. Grasshoppers will be collected using a sweep net or by hand, depending on their size and density. Ground-dwelling beetles will be collected in 1-gallon slick-sided pit traps (new 1-gallon paint cans) embedded in the soil, flush against the ground surface. Deer mice will be collected with live traps and dispatched by spinal-column separation or use of carbon dioxide (from dry ice vapor in a closed cooler). Starlings will be collected by hand from nest boxes and dispatched with carbon dioxide.

Table 3 Summary of the SFS-Phase I Tissue Collection Protocols 

| Specimen | | Collection | | |
|---|---|---|---|---|
| Species | Life Stage | Season | Method | Tissue |
| Black-tailed Prairie Dog | Juvenile nonlactating female or adult of either sex | Late Spring/ Early Summer | Live trap, rifle (.22 caliber) or shotgun 912, 16 or 20 gauge) | Dressed Carcass (+ Residuum from 10%) |
| Rabbit | Adult | Late Spring/ Early Summer | Rifle (.22) | Dressed Carcass (+ Residuum from 10%) |
| Grasshopper | Late Instar | Late Summer/ Early Fall | Sweep Net/ By Hand | Whole body (minus hardened hind legs); composited individuals |
| Ground-dwelling Beetle | Adult | Summer | Pit Trap | Whole body; composited individuals |
| Deer Mouse | Adult | Late Spring/ Early Summer | Live Trap | Whole body; same sex reserve sample if needed |
| Starling | Juvenile (immediately pre-fledgling) | Late Spring/ Early Summer | By Hand from Nest Box | Dressed Carcass (+ Residuum from 10%) |

To provide the best estimates of dose, samples will be collected from that portion of the organism that is typically consumed. However, to best estimate risk to the species being sampled, whole-body tissue concentrations are most useful. To avoid unassimilated contaminant concentrations, the gastrointestinal (GI) tract will be removed from all samples and discarded, except that insect samples will be left intact for practical purposes. Major amounts of fat associated with the intestinal tract will be removed and left with the sample. Samples minus the GI tract will still be termed whole-body samples.

Table 3 summarizes the sample collection methods, which are briefly described as follows. A prairie dog or rabbit sample will consist of the dressed carcass, i.e., the whole body minus head, skin and fur, and paws. Entire grasshoppers and beetles will be composited, except in the case of large grasshoppers. If large grasshoppers are collected, well-hardened hind legs will be removed since the legs are not consumed. One entire deer mouse will comprise a sample, and a reserve sample of the same sex will be collected when the sample weighs between 15 and 20 grams (g). (Mice weighing less than 15 g will not be collected.) Finally, a starling sample will consist of the dressed carcass, i.e., the whole body minus head, feathers (but not skin), tarsi, and beak. For those samples that consist of dressed carcasses, the removed body components of 10 percent of the samples will be analyzed as a second sample from each individual. These samples will ultimately be used to estimate a conversion factor between dressed-carcass and whole-body tissue concentrations. Appendix A provides further details on sample preparation and handling.

## 3.4 SAMPLE TYPES

Sample types were evaluated for all species to be collected, and individual sampling was recommended for starling, deer mouse, prairie dog, and rabbit. Deer mice will be captured and weighed in the field and those that are below an acceptable weight for analysis will be released. Composite sampling was recommended for insects to obtain adequate sample weight. As discussed below, composite sampling can be used to reduce the cost of laboratory analyses without reducing the level of accuracy for estimating the mean. For example, composite

sampling of prairie dogs in the bald eagle exposure area would reduce uncertainty in estimating the mean prairie dog tissue concentration and, therefore, the mean dose to the bald eagle; however, this approach would imply many more prairie dog samples and a larger impact on the prairie dog population compared to the laboratory analysis of each sample individual. Moreover, if samples are composited, information on individual variability that is useful for other analyses beyond those specified in the decision process presented in Section 2.5 would be lost. In particular, compositing does not allow estimation of the 90th percentile. Therefore, composite sampling was not recommended for those species to be evaluated for risk based on the concentrations in their tissues (i.e., prey species).

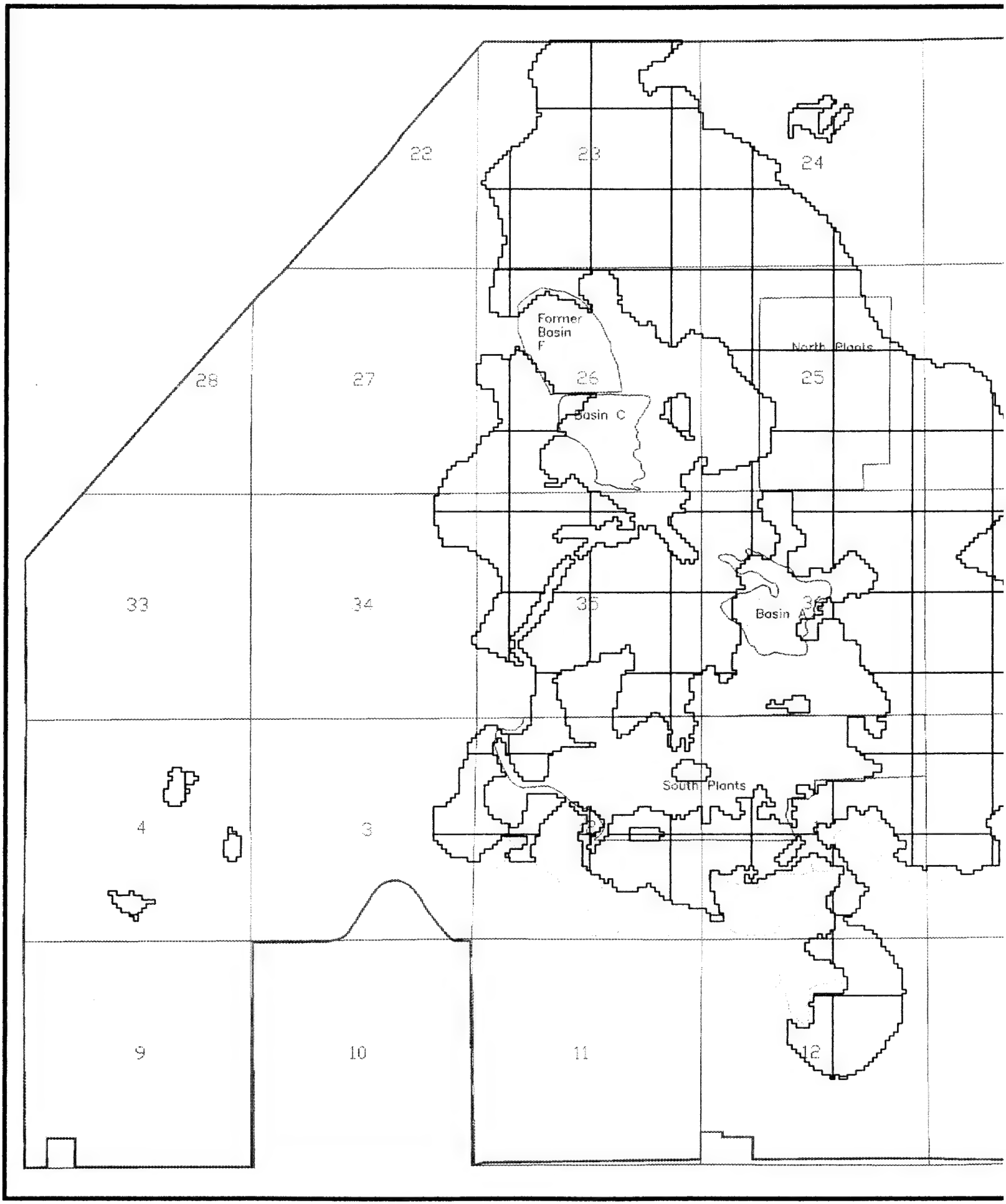
When composite sampling is unavoidable to achieve minimum sample weight, it will be conducted in different ways to produce different types of information. For insects, composite sampling will be conducted to give estimates of the spatial variation and the overall mean. The composite method makes use of multiple individuals from a given sample location or area by combining them into a single, localized sample that is representative of the mean value for that given location or area. The resulting set of local composite samples can be used to estimate the spatial variation in the mean, the overall mean, and uncertainty (i.e., confidence intervals) for the overall mean. One advantage of compositing local samples is that the sample variance is reduced (i.e., the variance of the number of composite samples is expected to be lower than the variance of the number of individuals) and that the resulting confidence intervals for the overall mean are narrower. Compositing by this method produces data that could be used as part of a study of collocated soil concentrations and BMFs. Local composites are optimal for estimating the mean BMF of animals living in different areas because variance due to individual physiology and behavior is averaged out to some extent, providing a stronger signal (less noise) for the relationship between soil and tissue concentration. The main disadvantage is that a larger number of individuals are removed from the population to achieve a given amount of information on spatial variability.

## 3.5 SAMPLE LOCATIONS

A random block sampling pattern was recommended for sampling in both the AOD and the bald eagle exposure area outside the AOD. Random block sampling realizes the benefits of systematic sampling (uniform coverage) and random sampling (adherence to the statistical assumptions on which the formulas for evaluation sample sizes and confidence limits are based) (Gilbert 1987). Shell Oil Company (Shell) conducted an independent evaluation and found that systematic sampling performed as well or better than purely random sampling, for sample sizes less than 60, in their simulation exercise for RMA (1994). The exercise assumed that spatial pattern and variability in tissue concentrations are directly related to pattern and variability in the soil concentrations. This result suggests that the increase in sampling uniformity that results from systematic or random block sampling is advantageous for estimating tissue concentrations on RMA. The rationale for selecting this common design is discussed further in Section B.2 of Appendix B. A description of the random block sampling pattern for the SFS-Phase I is provided below, as is a discussion of the impact of uneven biota distributions on allocation of samples according to area or biota abundance and a discussion of the methods for collecting samples within a block and for adjusting sampling pattern or data analysis for missing data.

### 3.5.1 Random Block Sampling Patterns for the SFS-Phase I

In general, random block sampling involves dividing an area into blocks of equal size (usually equal area) and taking one sample at random from each block. Partial blocks occurring at the edges of the AOD will receive one or no samples, with the probability of one sample equaling the area of the partial block divided by the area of a whole block. For all sampled species except prairie dog, the entire AOD will be divided into a number of blocks approximately equaling the recommended sample size for this area (Figure 5). For prairie dogs, distinct regions defined as having prairie dog towns in April 1993 will be assigned a fraction of the overall number of prairie dog samples where this fraction is proportional to the relative area of each region. Small regions may receive zero or one sample accordingly. Within a region, sample locations will be assigned according to the random block pattern or according to simple random sampling (if the area and number of samples are small). This approach will also be maintained for prairie dog samples within the bald eagle exposure area outside the AOD. These samples will be allocated


22
23
24
Former
Basin
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North Plants
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Basin C
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Basin A
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South Plants
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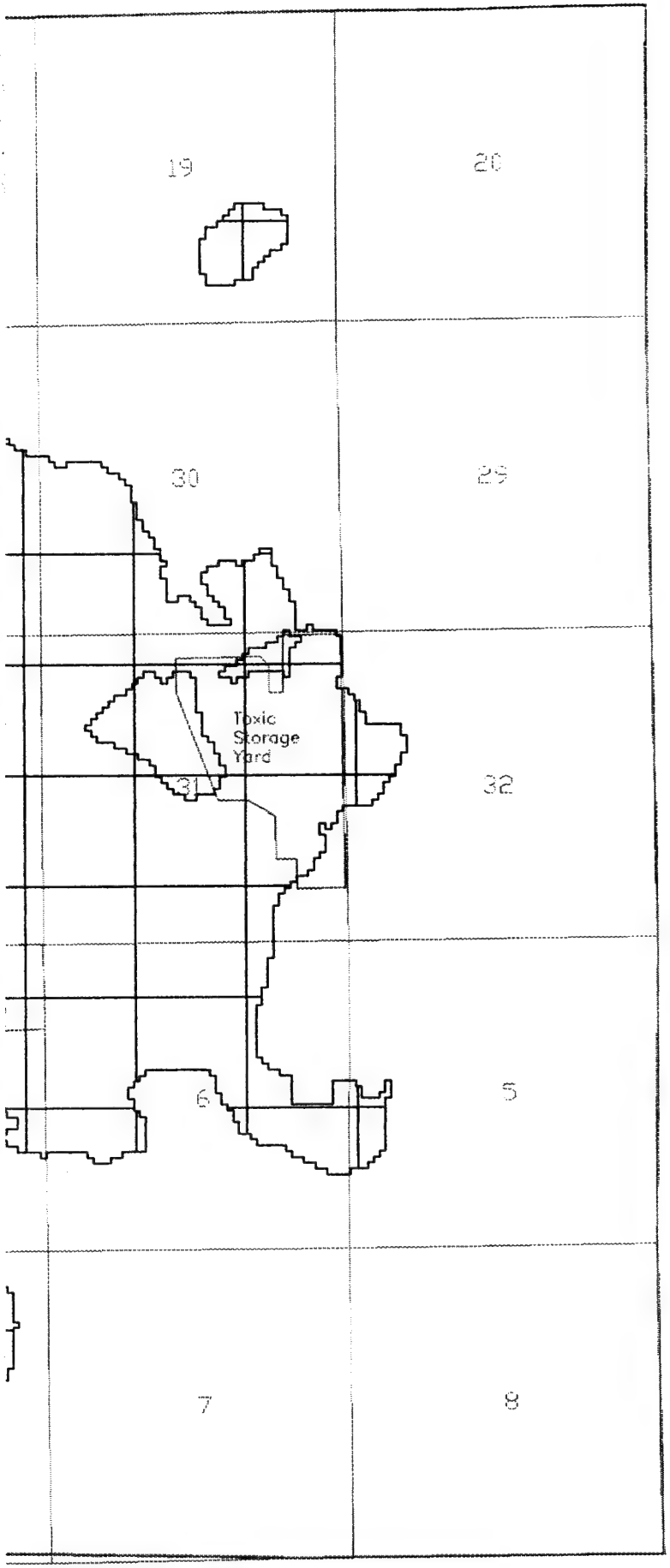


## Legend

Sampling Block

Lake

31 Section Number

Section Line

-N-

0 2000 4000

Scale in Feet

Prepared for:
U.S. Army Program Manager
for Rocky Mountain Arsenal

May 1994

Figure 5

Random Block Sampling Design for Approximately 50 Blocks. Prey Study Area

Rocky Mountain Arsenal
Prepared by: ENSERCH Environmental Corp

to prairie dog towns as described above. The sample sizes in the portions of the bald eagle exposure area within and outside of the AOD will not be designed to provide equal areal representation; therefore, the mean prairie dog tissue concentration for the bald eagle exposure area will be calculated based on areal weighting rather than by combining all samples with equal weight.

### 3.5.2 Allocation of Samples by Area or Biota Abundance

The allocation of samples to regularly spaced blocks ensures that equal areas within a study area receive equal weight in contributing to the study area sample mean. In general, this sampling objective, equal representation of area, is consistent with the stated goals of estimating risk for the specified AOD. Equal representation of area implies equal representation of different contaminant levels in the soil, i.e., the exposure of the sampled individuals is representative of the soil contaminant distribution across the study area. However, equal representation of area does not always imply equal representation of all members of the biota population since biota density may vary substantially across the study area. Therefore, sampling can be designed to estimate either the mean risk associated with a given area and contaminant distribution ("sample allocation according to area"), or the mean risk of the current biota distribution ("sample allocation according to biota distribution"). The random block concept applies to either case; blocks are defined to have equal area or equal prey density. Sample allocation according to area has the disadvantage that areas where a given species is not currently present cannot contribute biota samples to the data set. This may result in a risk estimate that is somewhere between that representative of the current soil contaminant distribution and that representative of the current biota distribution. Sample allocation according to biota distribution has two disadvantages: (1) it requires detailed knowledge of the biota spatial distribution, and (2) it results in risk estimates that are only relevant to the particular biota distribution observed in the year used for sampling design and may not reflect risk resulting from other potential and future biota distributions.

As stated above, sample allocation according to area is most consistent with the goals and methods of the Ecological Risk Characterization for the IEA/RC. Accordingly, this sampling approach will be followed for all species except prairie dog, although some deviations are likely

to occur as described below. For prairie dogs, knowledge regarding current distributions, and specific plans for management of this population, warrant the reduction of the sampling area to include only specified prairie dog towns. Therefore, as stated above, samples are allocated according to area within the prairie dog towns.

### 3.5.3 Selection of Sampling Location and Adjustments for Missing Data

In general, a sample will be taken from each block for each species. Within each block, the starting point for sample collection will be randomly selected. The starting point and actual sampling location will be recorded. If a species is not found during the sampling process within a given block, one of the following approaches may be used: ignore missing data blocks or derive an estimate for each missing data block based on data for the surrounding blocks. Both approaches will not induce bias provided the number of missing data blocks is relatively small for a given species and the absence of the species is not related to potential soil contamination (contamination levels high enough to preclude biota are not expected to occur in the study areas). In contrast, replacing missing data blocks with additional samples from already-sampled blocks creates a bias and should be avoided. The choice between these two alternative approaches depends on the spatial patterns of missing blocks and underlying contaminant distribution. Therefore, this choice is best made after sampling has been attempted for all blocks. If data are missing from numerous blocks, then the collection of additional samples may be desirable to increase the statistical power. The appropriate location of these additional samples will be based on the location of samples already collected and additional information such as the distributions of soil contamination and appropriate habitat for a given species. Statistical considerations such as spatial correlation and uniform representation of soil contamination will be considered in selecting locations of additional samples.

The process used to select sample locations must be flexible. It is emphasized that flexibility must be allowed in the process of selecting sample locations. The non-random distribution of biota within the AOD will inevitably introduce some bias into the sampling program, but the random block design will serve to minimize this bias for the purpose of estimating risk associated with a specific area and soil contaminant distribution.

## 3.6 NUMBER OF SAMPLES

Sample sizes were determined based on analyses by standard statistical methodology that relate sample size to the statistical power. Conclusions from the sample-size analyses are discussed in the following paragraphs.

Sample size for the prey species evaluation was evaluated based on two different statistical methods for the mean (assuming low skewness and high skewness) and two methods for HQ90 (parametric and non-parametric). The formulas used to investigate power and sample size when sampled prey tissues are used as doses to predator species are discussed in Appendix B. The calculations pertaining to power do not consider the loss of power resulting from using data with concentrations that are below certified reporting limits. Spreadsheets (Appendix B) allowed results to be calculated under a variety of assumptions regarding key parameters such as the projected variances of the populations.

For the prey evaluations, the selected sample size of 50 per species provides a moderate degree of statistical power, provided the standard deviation of the HQ is less than 0.75. Under this assumed standard deviation, there is at least an 80 percent chance of rejecting the null hypothesis (meanHQ > 1) if the true meanHQ is no larger than 0.73. In comparison, a sample size of 100 implies an 80 percent chance of rejecting the null hypothesis when the true mean is as high as 0.81 (assuming a standard deviation of 0.75).

In an independent simulation exercise, Shell (1994) found that the benefits of taking more samples diminishes substantially as sample size increased. This study also indicated that gains in estimation accuracy from increasing sample size beyond 50 were relatively low. This exercise was based on the assumption that the spatial pattern and variability in tissue concentrations at RMA are directly related to pattern and variability in soil concentrations.

From the perspective of estimating predator concentrations, samples should ideally be allocated to different prey items so that higher dietary fractions receive higher sample sizes. The reduced sensitivity of power to low-fraction prey items can be observed in the predator spreadsheets when

different sample sizes are entered for different species. These sheets show that sample size does not have a strong influence on power for the predator. Therefore, unequal sample sizes for prey items were not considered except where indicated by concerns regarding the biological impact of sampling.

Statistical power for the predator evaluations depends strongly on the mean of the prey tissue sample concentration in addition to its variance and the sample size. Preliminary analysis of the factors affecting power for the predator evaluations indicates that a threshold exists; if prey tissue concentrations are such that prey meanHQs are greater than about 0.1 to 0.2, depending on the predator, then the power to reject the null hypothesis for predator populations is very low for sample sizes up to at least 1,000 per prey item. The reason is that one of the additive terms contributing to the variance of the mean dose is independent of prey sample size. This term can be reduced through sampling only if a lower mean dose results. For such low prey meanHQs, the null hypothesis for both prey and predator evaluations can be rejected with relatively small sample sizes (n = 50 or less, in most cases). In short, the sample size analysis implies that the prey and predator null hypotheses can either be rejected with small sample sizes (if prey HQ is very low), or cannot be rejected with even very large sample sizes (if prey HQ is on the order of 0.2 or higher).

## 3.7 SUMMARY OF SAMPLING RECOMMENDATIONS

Sample Type:

AOD: Individuals for prairie dogs, rabbits, deer mice, and small birds; however, composites for insects.

Bald Eagle Exposure Area Outside the AOD: Individuals for prairie dog.

Sample Location:

Random block (Figure 5). Random blocks for prairie dogs will be selected based on a map of prairie dog townships.

Number of Samples:

The sample size plans for the AOD and the bald eagle exposure area outside the AOD are presented in Table 4.

The statistical objective in selecting sample sizes was as follows: sample sizes should provide adequate power to reject the null hypothesis whenever the true meanHQ is less than the action level of 1.0. For the prey evaluations, the sample sizes of 50 will provide adequate power for the meanHQ evaluations provided the true HQs have a standard deviation no larger than 0.75 and a true mean no larger than 0.73. This sample size implies a fairly wide confidence interval for HQ90 (0.18) if the standard deviation of HQ is as high as 0.5; however, this confidence interval was not incorporated into the decision process. The sample size for rabbits was set at 20 to reduce the impact on the rabbit population. The recommended sample size for beetles and grasshoppers, 25, assumes that these species will be pooled to estimate a single mean and standard deviation.

## 3.8 CHEMICAL ANALYTICAL PROGRAM

Biota tissues will be analyzed for seven OCPs including aldrin, dieldrin, dichlorodiphenylethene (DDE), dichlorodiphenyltrichloroethane (DDT), endrin, alpha-chlordane and beta-chlordane, plus two chlordane metabolites, oxychlordane and heptachlorepoxide. The basic technical approach will include Soxhlet extraction of the tissue followed by analysis using gas chromatography/electron capture detection (GC/ECD) or an alternate method using negative chemical ionization mass spectrometry (NCIMS). The GC/ECD analytical method will be based upon EPA's SW-846 method 8081, which uses capillary-column chromatography. For the GC/ECD methodology, second-column confirmation using a dissimilar megabore column will be used for positive identification of target analytes using both qualitative and quantitative verification. The NCIMS methodology may be a second possible analytical approach that could be used to meet the DQOs of the SFS-Phase I. The method is currently being tested by the U.S. Geological Survey (USGS) for application to the project. The technique uses selected ion monitoring (SIM) to qualitatively identify the compounds of interest and to provide the sensitivity needed for low levels of quantitation.

Table 4 Proposed Number of Samples



| Species | Sample Size | Approximate Contribution to Owl | Fractional Predator's Diet Kestrel |
|---|---|---|---|
| Area of Dispute | | | |
| Prairie dogs | 50 | | |
| Rabbit | 20 | 0.87 | |
| Deer mice | 50 | 0.13 | 0.69 |
| Starlings | 50 | | 0.12 |
| Beetles[1/] | 25 | | 0.095 |
| Grasshoppers[1/] | 25 | | |
| Total | 220 | | |
| Bald Eagle Exposure Area Outside the Area of Dispute | | | |
| Prairie dogs[2/] | 30 | | |
| Total | 30 | | |

[1/]Composite
[/2]Samples may be composited

Nominal sample size for both methods will be approximately 8 g and will be prepared and extracted as per existing Program Manager for Rocky Mountain Arsenal (PMRMA) biota methods. This involves grinding the tissues with dry ice to obtain a homogenate followed by standard Soxhlet extraction. Pesticide concentrations will be determined on a wet-weight basis with no attempt to dry the tissue prior to sample preparation. Percent lipids will be determined and reported for each sample with no correction being made to found concentrations. Aliquots of homogenate will be extracted within 1 week (7 days) of weighing. All sample homogenate remaining after the aliquots are made will be refrozen and saved for re-analysis, if necessary. Residual extracts will also be saved by the laboratory in refrigerated storage until approval is given by PMRMA for disposal.

At the same time the SFS-Phase I decision process is applied (see Section 1), the need to analyze the remaining sample homogenate for mercury will be reevaluated if pertinent results from the USFWS bio-monitoring program or other study indicate mercury is present in tissue at concentrations that may pose a potential risk.

The following DQOs relative to quantitation limits (target reporting limits, or TRLs) for the analytes of interest were established for the SFS-Phase I:

| Analyte | Tissue Concentration (µg/g) |
|---|---|
| Aldrin | 0.002 |
| Dieldrin | 0.002 |
| Endrin | 0.001 |
| DDT | 0.003 |
| DDE | 0.003 |
| Alpha-chlordane | 0.002* |
| Gamma-chlordane | 0.002* |
| Heptachlorepoxide | 0.002* |
| Oxychlordane | 0.002* |

* Chlordane-series compounds were set equal to the levels of aldrin and dieldrin since no other guidance was available.

For purposes of establishing analytical reporting limits, the Army's long-standing method of using certified reporting limits (CRLs) will apply. The CRL for a method is extracted from experimental data as described by Hubaux and Vos (1970). While the method detection limit (MDL) routinely used by EPA is determined using replicates at a single concentration, the CRL determination requires multiple concentrations be analyzed on more than 1 day, thereby providing a more realistic estimation of method capability and day-to-day variability. A specific comparison of MDL and CRL is provided in an article by Grant et al. (1991). Based on the established TRLs for the program, the tested concentration range for establishing the CRL will include 0.25 x TRL to 100 x TRL. The tested dynamic range may be reduced at the upper end if nonlinearity of instrumental response is observed, which would prevent accurate quantitation. Samples exceeding the upper range of the method will be diluted into the working range of the method. In addition, an MDL determination following standard EPA protocols will be conducted at some factor (possibly 5x to 10x) lower than the TRLs in an attempt to ascertain a possible MDL for the method. Standard Army convention for the reporting of found concentrations is for all values determined to be Less Than (LT) the CRL to be reported as such (LT). This demonstrates the emphasis needed for the proper and reasonable selection of study DQOs as required for assessment of potential effects to wildlife populations. For purposes of this study, EPA has requested that in the event the MDL for the method is determined to be lower than the corresponding CRL, a convention be set up to provide for the qualitative identification of positive analyte detections in the region <CRL but >MDL for the method. The quantitative value of such data is probably not satisfactory for purposes of the SFS-Phase I because the uncertainty of the data is great. However, any found value falling in this particular region of the method will be assigned an indicator that delineates whether the found value was closer to the MDL, i.e., lower half of the range, or closer to the CRL, i.e., the upper half of the range. This will simply provide a "frequency counter" to demonstrate the frequency of occurrence for this phenomenon. Specific proxy-value protocols for these values and for values less than the MDL must be developed and agreed upon by the RMA EA Technical Subcommittee using criteria consistent with EPA guidance for such proxy values. Alternative approaches considered for determining proxy values will include robust methods and established EPA options for proxy values. The possible development of more sensitive analytical methods that would use archived sample extracts or

homogenates may not be a viable option since the development of such analytical techniques in a time frame suitable for making decisions required for the SFS-Phase I is highly doubtful.

As required by the PMRMA Chemical Quality Assurance Plan, appropriate quality control (QC) samples will be used throughout the program to assess laboratory performance. This will include blind performance samples and duplicate samples from species such as prairie dogs and rabbits where abundant sample weight is available. All sampling and analytical methodologies will be fully documented, reviewed, and approved prior to initiation of field or laboratory efforts. Analytical method proficiency demonstration data will be available for review by the Parties as requested. The quantitation DQOs established above must be considered as goals, i.e., with no guarantee the experimental data will support any or all of target reporting limits, even though all attempts will be made to meet those objectives.

## 3.9 ADDITIONAL STUDY REQUIREMENTS

A detailed site-specific Sampling and Analysis Plan (SAP) (EBASCO 1994b) and Accident Prevention Safety Task Plan (EBASCO 1994c) have been prepared to implement the SFS-Phase I program. Elements of the SAP, such as sample collection procedures, are provided in Appendix A. In addition to having the chemical analyses performed by a certified laboratory, both the field sample collection and laboratory analysis will be performed in compliance with Good Laboratory Practices (GLP). GLP will include strict adherence to the performing laboratory's QA/QC management plan. Most of the procedures appropriate under GLP have been followed in past sampling and analysis of RMA biota and will be brought forward into the SFS-Phase I. The application of the GLP approach will be formalized in this sampling program.

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# APPENDIX A

# BIOTA FIELD SAMPLING PROCEDURES

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Figure

# INTRODUCTION TO APPENDIX A

# BIOTA FIELD SAMPLING PROCEDURES

This appendix provides general and species-specific recommendations for conducting field sampling activities. Those activities include a discussion of documentation as well as sample preparation, packaging, and handling procedures.

## A.1.0 GENERAL PROCEDURES

This section provides general procedures that are applicable to all biota being collected. Section A.2 provides species-specific details. Additional detail is provided in the SAP (EBASCO 1994).

### A.1.1 SAMPLING LOCATION

As described in the main text of this SFS-Phase I plan, a random block sampling pattern is to be used in both the AOD and in the bald eagle exposure area outside the AOD. Random block sampling realizes the benefits of systematic sampling (uniform coverage) and random sampling (adherence to the statistical assumptions on which the formulas for evaluation sample sizes and confidence limits are based) (Gilbert 1987).

The number of blocks to be sampled randomly reflects the number of samples to be collected for each species: if 50 samples are collected, 50 blocks will have been randomly sampled. The number of samples to be collected of each species was based on analyses by standard statistical methodology that relates sample size to statistical power. Conclusions from the sample size analyses were presented in the main text of this SFS-Phase I Plan and resulted in the numbers given in Table 4.

#### A.1.1.1 Selection

In general, random block sampling involves dividing a given study area into blocks of equal size (usually equal area) and taking one sample at a random location within each block. Partial blocks occurring at the AOD edges receive one or no samples, with the probability of one sample equaling the area of the partial block divided by the area of a whole block. In the SFS-Phase I, the random block approach shall be applied to each species being collected as described below.

When no sample is collected in a block designated for sampling, field team members should inform the Field Coordinator, but should not sample an undesignated block for a replacement sample. The decision on how to deal with missing samples will be made by the Technical and

Statistical Leads once most samples for a given species have been collected. If a species is not collected from several blocks, the missing data blocks will either be ignored or a value for the missing data blocks will be estimated from data for the surrounding blocks. This choice will be made after sampling has been attempted for all blocks because the choice depends on the spatial patterns of missing blocks and underlying contaminant distribution. If data are missing from numerous blocks, then the collection of additional samples may be desirable to increase the statistical power. The location for these additional samples will be based on the location of samples already collected and additional information such as the distribution of soil contamination and appropriate habitat for a given species.

The procedure for selecting sampling locations for each species shall be as follows:

- Prairie dogs—Prairie dog towns present as of April 1993 and within the AOD are to be divided into 50 blocks and prairie dog towns present as of April 1993 and outside the AOD are to be divided into 30 blocks. A geographic information system (GIS) will be used to first select the block size that best approximates the required number of blocks and then to draw the blocks. The probability of sampling fractional blocks is to be weighted based on fractional block size. Sample sizes do not provide equal areal representation of the AOD and sampled portion of the bald eagle exposure area. Therefore, data from these areas will be a really weighted to calculate the mean prairie dog tissue concentration for the eagle exposure.

- Rabbits—The AOD is to be divided into 20 blocks. The GIS will be used and the probability of sampling fractional blocks will be weighted as for prairie dogs.

- Deer mice—The AOD is to be divided into 50 blocks. The GIS will be used and the probability of sampling fractional blocks will be weighted as for prairie dogs.

- Starlings—The 13 groups of nest boxes established by the USFWS starling monitoring program in 12 sections on RMA are to be used as sampling locations. Based on a preliminary map, most nest boxes in four of these groups are clearly within the AOD. The 50 specimens are to be collected from the nest boxes within the AOD. The Field Coordinator is to check with the Technical Lead who will coordinate with USFWS to initiate the collection of starlings. At group locations where there are more occupied nest boxes than allocated samples, the boxes to be sampled will be randomly selected. If

USFWS has a conflict with the use of the nestlings at a randomly selected box, another box is to be randomly selected in the same manner.

- Beetles—The AOD is to be divided into 25 blocks. The GIS will be used and the probability of sampling fractional blocks will be weighted as for prairie dogs.

- Grasshoppers—Grasshopper collection will be at the same randomly selected locations used for beetle collection within each block.

A.1.1.2 Documentation

The sampling location for each species within the random block design shall be identified with an alphanumeric location identifier that will become part of the Site Identification number used in the Installation Restoration Data Management Information System (IRDMIS) data-tracking system and also by its x,y coordinates expressed in State Planar (STP) coordinate system units. For all species except starlings, the location identifier is a species-specific letter, and a four-digit location number (e.g., DB016 = deer mouse block number 16). The species-specific letters are as follows: P, prairie dog; D, deer mouse; R, rabbit; S, starling; B, beetles; and G, grasshoppers. In the location identifier for starlings, the "S" for starling is followed by a number designating the section of the group location and a two-digit number identifying the nest box at the group location (i.e., S0115 = starling group location in Section 1, 15th nest box).

There will be two sets of x,y coordinates associated with each sample, the randomly selected sampling coordinates that are used as starting points for sampling, and the coordinates of the actual sample collection location. The locations for the randomly selected sampling coordinates used as starting points for sampling are approximate for two reasons: (1) the closest available appropriate habitat within the block will be used for actual collection, and (2) this location will be either visually identified from a distance (to avoid disturbing the individuals being hunted) or paced off, depending on the species. If the closest available habitat within the block is not apparent from the starting point, a search toward the furthest boundary of the block will be initiated. If all boundaries are approximately equidistant, a randomly selected direction will be

used. The actual sample collection location is to be documented by a global positioning system (GPS) or standard survey method, and will have a precision error of ± 16 feet (ft) (5 meters [m]).

### A.1.2 SAMPLE COLLECTION PROCEDURES

Before an organism is collected, it should be accurately identified. The species-specific sampling procedures provide a brief summary of identifying characteristics, and field guides provide additional detail; the latter should be carried in the collection vehicle when sampling. The variety of taxa to be collected requires a variety of collection procedures. If firearms are used to collect specimens, the permits secured for each firearm must be in the possession of the gun-bearer at all times. The person registering the firearm is responsible for it at all times when at RMA. Other types of collection, such as trapping, have less potential impact on other personnel at RMA. However, all collections should be coordinated through the weekly USFWS Tuesday morning coordination meeting (0730, Building 111). This will help coordinate all activities ongoing at RMA.

To support the collection of each species, an RMA-wide map superimposed with the numbered blocks (or group nest locations), a map of each section containing sampling blocks for a species, a map of each block or group nest location (and for deer mice a map of the five-trap by five-trap trapping grid), a list of the randomly selected sample collection starting points for each block (or group nest location), and the materials for data recording are needed.

### A.1.3 DATA RECORDING

All field activities are to be documented according to very specific guidelines. Data from daily field and laboratory activities shall be recorded in data notebooks, with sample-specific data recorded on a sample tag, chain-of-custody (C-O-C) form, and field data form (FDF). Copies of these forms (Figures A-1, A-2, and A-3) are included at the end of this appendix. The information that must be included on these forms is identified below. Professional judgment should be used as to the recording of additional pertinent information in the Field Notebook.

### A.1.3.1 Data Notebooks

Data shall be written in waterproof black or blue pen in standard engineering notebooks. Several data notebooks are to be used: Field Notebooks carried by each collecting team and the Laboratory Notebook. In every data notebook, each page is numbered using a six-digit identifier. The first three digits refer to the book number and the last three to the page number. For example, 022-001 refers to book 22, page 1. Each of these entries must be initiated to indicate who is taking notes of the day's activities. The duration of each activity is recorded in military time. Incorrect entries are to be corrected by drawing single lines through the written material. Each such strikeout must be initialed. The Quality Assurance (QA) Manager is responsible for issuing data notebooks and recording information regarding their issuance (see Section 5 of the SAP; EBASCO 1994).

Information to be recorded in the Field Notebooks includes team members' names, starting and ending time, activity, location of work, sites worked in or near, species sought, and general observations throughout the day. Recorded information about the locations where the target species are being sought for collection must include block number and information about the distribution of appropriate habitat, the species, and the pattern of attempted collection. The following is an example of an entry:

> "In B140, prairie dogs were observed only in the SE ¼, about 50 ft north of the random starting coordinates (Sx=2189497, Sy=180717); sample #0912-05/24/94-MJ-CYLU was collected by rifle and a spike was driven into yellow flagging at the collection location (approximate coordinates of Tx=218950, Ty=180780), which is about 600 ft E of the intersection of 7th and E Streets."

Note that distance on the ground should be recorded in ft because the STP coordinates available for RMA maps are in feet. The GPS reads only in meters and will need to be documented in meters and later converted. Therefore, be very careful to always record the units with distance measurements. All other measurements are in metric units.

Additional data can be recorded in the field notebook as appropriate. Such information might include unusual physical characteristics, an estimate of specimen age based on the characteristics observed (plumage, pelage, etc.), relative percentages of visually distinct types as described for grasshoppers, data on any photographs taken, (photographer's name, date, roll number, frame number, location and subject of photographs), whether any of the species to be collected at a given assigned sample location were absent, and whether the sample was collected within the boundaries of the sample block and within the species-specific sampling area. If collection outside the sampling area was necessary, the information (contamination data, habitat, etc.) used to choose the area of further search should be summarized.

Sample tag numbers are assigned by the computer. For completed samples, the final sample tag numbers should all be preceded by a "B". If a specimen is incipient (e.g., it is a potential replacement sample, or weighs too little to comprise a complete sample by itself), it is preceded by an "F" number since it is recorded by the computer program as a fortuitous sample. The "F" numbers are also assigned consecutively, and should be converted to "B" numbers when a sample is identified as complete.

The Laboratory Notebook shall be used to record the sample tag numbers of the samples processed for the day (and the temporary sample identification number assigned in the field, as discussed below) and any difficulties or unusual circumstances encountered during sample preparation for the day. Data sheets that contain necropsy information for each vertebrate sample prepared as well as taxonomic information for each insect sample are to be cross referenced to the Laboratory Notebook. The necropsy of each vertebrate sample shall be conducted according to the protocols provided in the SAP (EBASCO 1994) and its results recorded on preprinted data sheets that include data fields for the following information: sample tag number, species, date, and necropsy data. Whether the necropsy results were normal or abnormal should be noted in the Observations and Abnormalities section of the FDF, which should also cross reference the Laboratory Notebook number and page and the data sheet on which any abnormal results are described. For both grasshopper and beetle samples, the taxa included in each sample should be

identified and listed with the sample tag number on preprinted data sheets that are also cross referenced to the Laboratory Notebook number and page.

A.1.3.2 Sample Tag, Field Data Form, and C-O-C Form

An FDF, sample tag, and C-O-C form shall be completed each time an individual sample is processed, whether the sample is intentional or incipient. With the exception of the sample tag number (as noted below), all three sheets are completed identically for intentional and incipient samples.

The sample tags (Figure A-1), C-O-C forms (Figure A-2), and FDFs (Figure A-3) are similar to those that were used for the Biota Comprehensive Monitoring Program (CMP) (RLSA 1992). These data sheets are to be filled out in the laboratory using a laptop computer. Once entered, this information is output on preprinted five-ply forms and checked by a second person before being attached to the sample (sample tag) or placed in the appropriate folder (FDF and C-O-C form) in the freezer at the end of the day.

On the FDF, sample tag, and C-O-C form, the following data shall be recorded:

- Ten-digit Site Identification (ID) number (as described above and below)
- Sample tag number as a consecutive number with the format "B0001...B9999" as described below
  - — If the sample tag number is an "F" number rather than a "B" number, it should be written in the margin so that the "B" number can be put in the proper field when the conversion occurs
  - — Where multiple tissues from the same specimen comprise several samples, the format "B001A...B999Z" should be used with the letter designations as defined below
- Site type, always filled in "BIOL"
- Collection date in a six-digit format (i.e., month/day/year)
- Collection time using military time (i.e., 0001 to 2400 hours)

- Technique, always filled in with a "G", defined in the U.S. Army Environmental Center (USAEC) users' manual as a grab sample, but used here primarily as a space filler since the actual information will be filled in on the FDF

- Species as the four-letter code from the data sheet, defining starling as STVU and jackrabbit as LECA when needed under "other" (on the sample tag, common names should be spelled out completely)

- Tissue as the one-or two-digit code from the data sheet (C-O-C form only)

- Samplers' names as hand-written signatures

All entries must be right-justified. The format by which the 10-digit Site ID number is compiled must never vary. All Site ID numbers must be compiled according to the following protocol:

- Digit 1—Always "B" for biota.

- Digits 2 and 3—Always "SI" to signify SFS-Phase I sample data.

- Digit 4—Always one of the following: P for prairie dog, R for rabbit, D for deer mouse, S for starling, B for beetles, and G for grasshoppers.

- Digits 5, 6, 7, and 8—Always serve as the location identifier. Digit 5 is always a "B" or an "A" (except for starlings), indicating that the following three digits are a block number. (The "A" was used only with prairie dog block numbers in the bald eagle exposure area and outside the AOD.) It should be noted that three digits should always be used for the block number, even if the number is less three digits in length. For example, block #5 would be indicated as "005". When Digit 4 is an "S" (i.e. for starlings), then Digits 5 and 6 are a number designating the section of a group nest location and the following two digits are a nest box number.

- Digits 9 and 10—Always indicates the collection year (e.g., "94").

Any new acronyms shall be defined in the Field Notebook the first time they are used.

Samples are also identified by the pair of x,y coordinates for the location where the sample was collected. As noted above (Section A.1.1.2), the x,y coordinates that identify the collection starting location within a block are to be randomly selected and listed together with the block number in a table, which also lists three additional x,y locations to be used in sequence, if

needed. These starting x,y coordinates (Sx, Sy) are to be recorded in the Field Notebook as the approximate (i.e., paced or visually scaled off, but not surveyed) starting point. The location of actual sample collection is to be marked in the field with a metal stake hammered flush with the ground and with engineering flagging. It must also be marked on the block-specific map with an "X" that will later be surveyed to establish the precise (to $\leq \pm 5$ m) coordinates (Fx, Fy). In the interim, the actual sampling location should be described in the field notebook or on the annotated map in sufficient detail that it can be found by a person who has not seen the site previously. Tentative x,y coordinates (Tx, Ty) for the "X" can be read from the map and noted in the field notebook when the sample is collected if these tentative coordinates will aid in relocating the stake and flagging at the collection location. Surveying shall be accomplished with GPS unless it proves inefficient or inaccurate, in which case traditional survey techniques are to be substituted.

When more than one sample comes from a specimen because residual tissues (i.e., tissues removed to prepare a dressed carcass—skin, head, and feet for mammal; feathers, beak, and tarsi for birds) are also collected, the numeric portion of the sample tag number should be the same for all samples from that specimen. Individual samples are differentiated by the addition of an "R" to designate the residual sample. For cottontails, the sample tag number with an added "H" to designate a head saved for taxonomic identification shall accompany the foil-wrapped head which will be stored in the freezer.

The species name and tissue type must be spelled out on the sample tag to guide the laboratory's sample preparation since these data do not go on the laboratory's Chemical Data Coding Form.

Samplers' names initiate the C-O-C process. It is important that the sampler's signature be used; names should be printed underneath if the signature is not legible. Samplers then sign the C-O-Cs over to the necropsy person, who signs the samples over to the freezer.

The FDF has a standard allocation of fields that must be used for all samples. The FDF contains most of the data on the sample tag and C-O-C forms (site identification, sample tag number, collection date, and species and tissue, but not site type or technique); these data are filled in on the FDF in the same format as they were entered on the sample tag and C-O-C form. All of these forms are prepared using the laptop computer.

The FDF also contains some additional items of general information, including the following:

- Sample location, recorded as county, range, township, section, and quarter section
- Collection coordinates, marked with an "X" on the map accompanying the FDF; the tentative STP location coordinates for this "X" are read from the map as northing and easting coordinates (Tx, Ty) and written in the field notebook; the final STP coordinates (Fx and Fy) from surveying are to be written by hand in the FDF data boxes after surveying is complete
- Habitat type, recorded from the vegetation map for RMA in the base trailer
- Collection method, life stage, and sex are all selected from among the categories listed on the FDF
- Sample weight, recorded as weighed to the nearest 0.1 gram (g), except where weight exceeds 100 g, round to the nearest whole number

There is also a section on the FDF for data specific to the various organisms being collected. In the first species-specific field, the nest box number is to be recorded for all starling samples, or the number of the trap in which the deer mouse sample was caught is recorded. For all composite whole-body samples of animals (grasshoppers and beetles), the number of individuals in the sample is recorded. If a reserve deer mouse is collected because the mouse collected in the block weighs between 15 and 20 g, the reserve mouse must be given an "F" number (not a "B" number) on a separate set of data forms. Soil type from the soil map of RMA in the base trailer is to be recorded for deer mice and prairie dogs. The number of readily recognizable taxa in the sample is also recorded for grasshoppers and for beetles. The area swept (in square meters) is to be recorded for grasshoppers. Note that a list of the taxa in each grasshopper and beetle sample should be recorded in the Field Notebook.

Field entries must be right-justified on the data sheet, or be aligned by decimal point, and recorded in the units indicated on the FDF. If the weight of the sample allows, a specimen of each type of grasshopper and beetle collected during the summer should be saved in ethanol as a reference collection of the taxa that were sampled on RMA. One specimen of at least the most common taxa of each species must be saved.

A.1.3.3 Sample-Specific Data Recording

In the field at the time of sample collection, a temporary sample identification number that consists of the collection time and date, the collector's unique initials, and the species acronym (e.g., 0900-5/24/94-MJ-PEMA), must be assigned and remain with the specimen as well as recorded in the Field Notebook. The temporary number will be converted to a final sample tag number in the laboratory when the sample is prepared. The following information should also be recorded in the field notebook: block number (or group location letter and nest box number), Sx and Sy and Tx and Ty coordinates, and collection method. For grasshoppers, the size of the collection area must also be recorded in square meters. A map identified with the temporary sample identification numbers should also be annotated for each sample as follows:

- Prairie dogs—On the block-specific map, mark the location where an individual was first seen with an "S". This mark should be made in the field and, if possible, prior to stalking the animal. Once collected, the actual sample collection location should be marked with an "X".

- Rabbits—On the block-specific map, annotation should be as for prairie dogs.

- Deer mice—On the block-specific map, mark the initial corner of the trapping grid with an "X"; the map of a standard five-trap by five-trap grid should be annotated by circling the trap in which the specimen was caught as the actual sample collection location and noting any deviations in layout of the trapping grid.

- Starlings—On the group-location-specific map, circle the nest box from which the specimen came.

- Beetles—On the block-specific map, mark the location of the pit trap with an "X" and note the location of any lawn edging used as trap wings.

- Grasshoppers—On the block-specific map, mark the center of the circle being swept with an "X" and draw an outline of the area being swept.

These and other data will be recorded on the sample tag, FDF, and C-O-C form, as appropriate, using the laboratory laptop computer. Maps will be available in the laboratory to determine habitat type and soil type.

At the end of the day when a sample is collected, the data forms and sample preparation must be completed, sample tags taped to the sample with strapping tape, C-O-C forms for both intentional samples and incipient samples signed over to the freezer and placed in separate B-C-O-C and F-C-O-C files either in the freezer or filing cabinet and the freezer and filing cabinet locked. The C-O-C forms must remain in the freezer with the samples until they are signed out of the freezer for shipment and they must accompany the samples during shipment. Completed FDFs must be placed in either the BFDF files or the FFDF files in the freezer or filing cabinet. The FDFs for incipient samples will still be lacking final "B" sample tag number that shall be assigned when the incipient sample is converted into an intentional sample. At the end of the sampling day, four copies of the maps associated with completed intentional samples should be made. These copies should be kept with the five-ply FDF. Once the data are complete and the samples are being shipped to the laboratory, the original map(s) and one copy should be attached to the original and first copy of the FDF, and together with two copies of the sample tag, a copy of pertinent field notes, and a transfer file of the data entered into the computer should be sent as a data packet to the QA Manager. Another FDF/tag/map set should be placed in files for completed samples in the biota filing cabinet. When the samples are shipped, one of the two remaining FDF/tag/map sets goes to the RMA Shipping Coordinator and the other goes to DP and Associates (DP). To ship samples, the C-O-C form is signed by the person packing the cooler as well as by the RMA Shipping Coordinator, at which time the back three copies are removed, two for the QA Manager and one for DP (The remaining two copies are to be signed over to Federal Express, or other carrier, by the RMA Shipping Coordinator, who keeps one copy

while the carrier signs the original C-O-C form over to the contract analytical laboratory, which will incorporate it into the data packet for the sample.)

In the office, the QA Manager should log in the data packet (2 FDFs on top, then map and field notebook pages) and check it for completeness and accuracy immediately. The QA Manager then logs out the second FDF to the Project Data Manager, who checks the transfer file against the FDF and initiates its data tracking system then returns the second FDF to the QA Manager to verify completion of data entry. Meanwhile, the QA Manager logs out the original FDF and associated map(s) and field notebook pages to the archive files and the copy (minus the second FDF) to the QA file. The final column in the QA log verifies the completion of data QA/QC and entry by documenting the return of the second copy of the FDF to the QA file.

### A.1.4 SAMPLE HANDLING

Some of the organisms die as a result of collection, while others need to be dispatched. Warm-blooded organisms that are still alive when captured are to be dispatched by cervical separation (deer mice) or placed in a cloth bag (starlings) or left in the live trap (rabbits and prairie dogs) and suspended in an asphyxiation cooler reserved for this purpose and containing enough dry ice or piped in carbon dioxide to produce an atmosphere rich in carbon dioxide. Alternatively, rabbits and prairie dogs may be dispatched with a pellet gun. Insects are to be dispatched by placing their collection container in the freezer.

Collected specimens must be prepared for shipment to the analytical laboratory. This involves selecting the particular tissues to be sampled, preparing the sample, and packaging the sample. While details of tissue selection and sample preparation are provided below for each species, a number of generalizations can be made.

Protocols for sample handling during preparation, packaging, transportation, and analysis are designed to prevent extraneous sample contamination. Samples can become contaminated during transport from the collection location to the preparation laboratory, during sample preparation,

during sample transport to the analytical laboratory, and during laboratory analysis. The following measures shall be used to avoid extraneous sample contamination:

1. In handling the freshly collected sample, make sure your hands are clean or wear clean cotton gloves and put the sample in a clean cloth bag (prairie dog, rabbit, starling, deer mouse), or clean glass bottle (grasshoppers, beetles). Do not use plastic bags or rubber gloves. Have the gloves and bags washed by a commercial laundry between uses. In addition, have the insect nets washed between use at different sample sites, and decontaminate the Sherman traps and shovels (by washing in a trisodium phosphate [TSP] solution or other laboratory cleaner, rinsing, and air drying) between use at different sample sites. Before new live traps and shovels are used the first time, they should be rinsed with hexane and then washed.

2. In preparing samples, wipe the sample preparation area with a TSP solution, rinse it with deionized water, and dry it with paper towel. To minimize the cleaning that is required, cover the cleaned preparation area with a clean square of cardboard before beginning to prepare a sample. Rinse all new metal equipment (e.g., scalpel blades, knives, etc.) with hexane, wash it with TSP solution, rinse with deionized water, and air-dry it before its first use. Subsequently, between specimens, this equipment needs only to be washed with a TSP solution, rinsed with deionized water, and air dried. Aluminum foil should be rinsed with hexane on the shiny side and folded into packets with the rinsed sides touching. Samples or clean equipment are then placed on the rinsed side. Unless glass bottles are precleaned by the laboratory, they should be washed with a TSP solution, rinsed, and air-dried before each use, including the initial use.

3. Clean tools must be available in the sample preparation area before each day's field effort. Used tools should be washed at the end of the day. Try to have enough clean equipment to prepare several specimens without washing. Wash all used equipment at once when you are through with sample preparation. Similarly, try to pre-rinse a quantity of aluminum foil so foil does not need to be prepared for each sample.

4. Keep debris from sample preparation in the plastic-lined garbage can in the sample preparation area. This waste material must be disposed on a <u>daily</u> basis.

Immediately after preparation, specimens in the form they are to be packaged and analyzed (e.g., gastrointestinal tracts are removed, feathers, beaks, and tarsi are removed, etc. as indicated below for the individual species) are weighed. Each sample should weigh at least 20 g. This will not be a problem for prairie dogs or rabbits, and probably not for starlings. The species-specific protocols regarding stipulations on weight for deer mice, grasshoppers, and beetles should be consulted for reference. Before specimens are weighed, a clean weighing paper, the glass bottle,

or the hexane-rinsed packaging foil must be placed on the scale platform and tared. Weighed samples are then packaged in TSP-washed glass bottles or wrapped in two sheets of hexane-rinsed extra-wide, heavy duty aluminum foil of an appropriate size. Packaged samples are then frozen for at least 24 hours before shipping. Shipping coolers should contain a minimum ratio of 1 pound dry ice per 3 pounds of frozen sample. A cooler should be moderately full prior to shipping. Shipping is the responsibility of the RMA Shipping Coordinator, who should be notified at least 1 day ahead of an intended shipment and the anticipated number and size of coolers. Because frozen samples will be placed in coolers that remain taped and under C-O-C protocols during shipment, extraneous sample contamination during shipping is not anticipated.

Protocols addressing prevention of extraneous sample contamination at the laboratory are addressed by the laboratory's QC plan. They are incorporated here by reference.

## A.2.0 SPECIES-SPECIFIC PROCEDURES

### A.2.1 BLACK-TAILED PRAIRIE DOG

Collection:

Black-tailed Prairie Dog (*Cynomys ludovicianus*)

Associated with bare 1- to 2-foot-high mounds on short-grass prairie; yellowish animal with terminal third of short tail black; ears small and belly pale buff or whitish. (White-tailed prairie dog: found in mountains, tail white.)

| Collection | | | | | |
|---|---|---|---|---|---|
| Timing | Locations and Numbers | Coordination/ Permits | Method | Age | Tissue |
| Late Spring/ Early Summer | Random locations within each of 50 AOD blocks and each of 30 blocks outside the AOD but inside the eagle exposure area | None on RMA | live trap or .22 rifle | Adult and Juvenile | Dressed carcass composed of whole prairie dog except for hair, skin, head, feet, and GI tract; the removed tissues (except the GI tract) should be saved as a separate sample identified by the same sample tag number (but with an R added) |

- In the late spring, collect samples at the periphery of the AOD or eagle exposure area, preferentially collecting nondispersed 1-year-old juvenile females (that are not lactating) because they do not disperse in early spring. Therefore, they are less likely to have been exposed outside the AOD. Later in the season and in other areas, collect adults to minimize having samples that may have just moved to the collection location and that may still be growing (and therefore reflect growth dilution), and to maximize the time period for collection. Collect adults only after young of the year are above ground and well grown.

- Obtain live traps from USFWS.

- Pre-bait locations where prairie dogs are to be trapped with alfalfa pellets for about 3 days before setting live traps.

- Use live traps to collect juveniles, so they can be checked for sex and breeding status. Collect nonlactating female juveniles. Female juveniles do not disperse in the spring as males do and should therefore be collected preferentially; yearling females occasionally breed, so check to ensure the female is not lactating before collecting it.

- Use live traps to collect adults if trapping is an effective and efficient means of collecting adults; if not, resort to firearms.

  Note: When firearms are being used, a USFWS staff member intends to accompany the collecting team; make sure you coordinate with USFWS to arrange a meeting place and time.

- When collecting with firearms and an appropriate juvenile cannot be collected in a block (i.e., not present, cannot be differentiated, or may have just moved in), collect an adult after trying for a reasonable time to select and collect a juvenile.

- If appropriate prairie dog specimen is not present (or lost) within shooting range or a direction that is safe to shoot from the random starting location, walk toward the closest observable prairie dog aggregation; search within block until collection possibilities are exhausted.

- Minimize the likelihood of a shot specimen entering burrow. If the specimen is only wounded once it is shot or has been live trapped, place it in the asphyxiation cooler or dispatch it with a pellet gun.

- If specimen is lost down a burrow, collect a second specimen from same random location (if available).

- Record the shooting or trapping of any USFWS-tagged prairie dog (both of which are permitted) in your Field Notebook, including information on the tag, and make an extra copy of data pertinent to this specimen for USFWS.

  Note: Plague has been documented on RMA (winter 1988–89); take necessary precautions (taped sleeves, visual inspection) to prevent the fleas that are often on these prairie dogs from biting. While use of insect repellent is undesirable because of the potential for sample contamination, the Element Manager is investigating the acceptability of various brands of repellant and protocols for their application. Check with the Element Manager before using any repellant.

Sample Preparation:

- Detailed procedures for skinning and performing a necropsy on a prairie dog are provided in the SAP; following necropsy, the sample is comprised of a dressed carcass, which is defined as a whole prairie dog minus the head, skin, feet, and gastrointestinal tract.

- Save removed body parts (i.e., head, skin, and feet but not gastrointestinal tract) preparing them as a residual sample, i.e., appending an "R" to the original sample number (e.g., B0458R). (Since only 10 percent of the residual samples are to be analyzed, i.e., five specimens within the AOD and three specimens within the eagle exposure area, randomly select the blocks from which residual samples are to be saved before collecting the first prairie dog).

- Weigh samples.

Packaging Procedure:

- Double-wrap the sample with hexane-rinsed aluminum foil; fill out the sample tag and C-O-C form; tape sample tag on outside of sample; put C-O-C form into the envelope in the tray of the cooler in which samples are placed.

- Dispose of gastrointestinal tract in garbage can during day, transferring all refuse to the biota garbage can at end of day.

Recording Details:

- Record all general data.

- Record any unusual physical characteristics.

- Describe the soil type.

- Identify the block number.
- Indicate where the specimen was first observed and where it was ultimately collected (mark on map).

## A.2.2 RABBIT

Collection:

Desert Cottontail (*Sylvilagus auduboni*)

Pale grayish washed with yellowish over much of the body; ears relatively large (3–4 in.). (Eastern cottontail: feet whitish, nape patch rusty and distinct, larger, but with 2.5–3 in. ears. Mountain cottontail usually not below pine zone in mountains.)

Eastern Cottontail (*Sylvilagus floridanus*)

Similar in general appearance to both desert and Nuttall's cottontails, but in general is larger, darker in color, and has relatively shorter ears than either of these species; patch on throat and chest bright rusty brownish (patch on desert cottontail described as orangish); see other comments under desert cottontail.

Black-tailed Jackrabbit (*Lepus californicus*)

Dorsal color grayish black and ventral color white. Black dorsal stripe extending from the tail onto rump; ears blackish on outer tips; Young have a pronounced white spot on the forehead (avoid these if possible); ears from about 100 to 130 mm; hind foot greater than 105 mm in adults; interparietal distinct and not fused to parietals (to distinguish from cottontails).

| Collection | | | | | |
|---|---|---|---|---|---|
| Timing | Locations and Numbers | Coordination/ Permits | Method | Age | Tissue |
| Late Spring/ Early Summer | Random locations within each of 20 blocks | None on RMA | .22 rifle; 12, 16, or 22 ga. shotgun, or live trap | Adult | Dressed carcass composed of whole cottontail except for hair, skin, head, feet, and GI tract; the tissues removed from the dressed carcasses (except the GI tract) should be saved as a separate sample identified by the same sample tag number but with an R added |

- Collect either species of cottontail (desert or eastern) or black-tailed jackrabbit, whichever is encountered first; pool the results from both species to estimate risk to owls. The species that is actually collected should be identified on the FDF as can best be determined from external characteristics. Differentiation between desert and eastern cottontails is based on skull characteristics and ear measurements and is very difficult. Further, there could be a hybrid situation on RMA, since specimens from 1988 had ear lengths within the range of eastern cottontails, yet their skulls, when compared to the USFWS reference collection were like those of desert cottontails. Therefore, try to avoid

shooting cottontails in the head; freeze the skull separately, tagging it with the same sample number and adding an "H" to indicate the sample is a head (e.g., B0458H).

- Initially, use live traps to collect rabbits, obtaining live traps from USFWS.

- Three days before the live traps are to be set, pre-bait the trap locations with alfalfa pellets. The traps should be left open over night and checked in the morning.

- If the live-trap method proves to be inefficient or ineffective, select either rifle or shotgun as the collecting method, depending on professional judgement regarding the most effective method in the habitat at the collection location.

- If an appropriate specimen is not present within shooting range or in a direction that is safe for shooting from the random collection location, or if an appropriate habitat is not present for trapping at the random collection location, walk toward closest observable appropriate habitat; search within block until the possibilities are exhausted.
- If the specimen is only wounded once it is shot or has been live trapped, place it in the asphyxiation cooler or dispatch it with a pellet gun.

Sample Preparation:

- Follow the detailed procedures for skinning and performing a necropsy on a cottontail/jackrabbit provided in the SAP; following necropsy, the sample is comprised of a dressed carcass, which is defined as a whole cottontail/jackrabbit minus the head, skin, feet, and gastrointestinal tract.

- Save removed body parts (except GI tract), preparing them as a residual sample, i.e., appending an "R" to the original sample number (e.g., B0458R). (Since only 10 percent of the residual samples are to be analyzed, i.e., two from the AOD, randomly select the blocks from which samples are to be saved before collecting the first rabbit.)

- Weigh samples.

Packaging Procedure:

- Double-wrap the sample with hexane-rinsed aluminum foil; fill out the sample tag and C-O-C form; tape the sample tag on the outside of the sample; put the C-O-C form in the tray of the cooler in which samples are placed.

- Dispose of gastrointestinal tract in garbage can during day; transferring all refuse to the dedicated biota garbage can at end of the day.

Recording Details:

- Record all general data.
- Record any unusual physical characteristics.
- Identify the block number.
- Indicate where the specimen was first observed and where it was ultimately collected (mark on map).

## A.2.3 DEER MOUSE

Collection:

Deer Mouse (*Peromyscus maniculatus*)

Color ranges from pale grayish buff to deep reddish brown; tail is always sharply bicolor, white below, dark above. (Other likely species, based on range of occurrence, are northern grasshopper mouse with gray or pinkish cinnamon color above and white beneath, short fur, and short, white-tipped tail; house mouse with scaly, sparsely haired tail about same color above and below; plains and western harvest mice, which are small, brownish and similar to a house mouse, but with a distinct groove running down the length of the front teeth. This assumes that other rodent families such as the following will not be confused with the deer mouse: pocket gophers with external cheek pouches; exposed incisors; large curved front claws and a short tail sparsely covered with hair; pocket mice, kangaroo mice and kangaroo rats with fur-lined cheek pouches, weak front feet, strong and well-developed hind feet and legs, and tail as long as or longer than the head and body; voles with small ears, short tails, and a chunky build; and jumping mice with extremely long tails and large hind feet, but without external cheek pouches.)

| Collection | | | | | |
|---|---|---|---|---|---|
| Timing | Locations and Numbers | Coordination/ Permits | Method | Age | Tissue |
| Late Spring/ Early Summer | Random locations within each of 50 blocks | None on RMA | Sherman live trap | Adult; do not collect females that are obviously lactating | Whole bodies to provide at least a 20 g sample. If only individuals between 15 and 20 g are collected after two nights, collect two individuals (of the same sex if possible); prepare one as the sample with a "B" sample tag number; keep the other as an incipient sample with an "I" number. Individuals weighing less than 15 g will be released alive. |

- Establish a five-trap by five-trap grid with 33-ft (10-m) spacing and the randomly selected starting location at one corner of the grid, but modify this protocol by professional judgment so that traps are placed in likely habitat and the grid remains within block boundaries; document any deviations from the protocol.
- Note that USFWS has ear-tagged some deer mice in areas marked on a map that will be in with the deer mouse maps; if the random starting location is in these USFWS trapping

areas, select the second random starting coordinates; if an ear-tagged mouse is trapped anyway, do not collect it unless it is dead in the trap; provide USFWS with copies of all data pertinent to such a mouse, either trap/release and tag information for a released mouse, or a full data packet for a dead individual that was collected.

- As traps are checked, put the specimen and a tag including trap number into clean cloth bag. Since the goal is to sample individuals (i.e., one individual weighing at least 20 g per sample), do not dispatch the individuals in the bags until all traps have been checked and all individuals have been weighed with the Pesola scale. Save the heaviest mouse for the sample. If it weighs less than 20 g but more than 15 g, save a second individual of at least 15 g (and of the same sex if possible). Other mice should be released at the trap location where they were caught.

- Collect nonlactating individuals (to avoid known dilution of contaminant concentration by lactation, have sample type be as homogeneous as possible, and avoid unnecessary death of young).

- Weight of at least 15 g has precedence over lactation; therefore, if the only sample weighing at least 15 g is lactating, collect it anyway.

- Place the cloth bags containing the mice to be dispatched in the asphyxiation cooler; remove them from cloth bag before packaging. An alternative to this method is to squeeze just behind the head of the mouse (this can be done through the cloth bag) until the spinal column separates and/or the mouse asphyxiates. This eliminates the logistical difficulty of maintaining dry ice, but is more difficult to perform for some persons.

- If deer mice of sufficient weight (20 g) to be collected have not been trapped at the random sampling location after 2 nights, collect a lighter-weight specimen if it is at least 15 g, but also collect a second deer mouse as a reserve sample (same sex, if possible) from the location trapped.

- If no deer mice have been trapped at the random sampling location after 2 nights, move to a second randomly selected location within the block.

Sample Preparation:

- Follow the detailed procedures for performing a necropsy on a deer mouse provided in the SAP; following necropsy, the sample is comprised of a whole body, which is defined as a whole deer mouse minus the gastrointestinal tract.

- Weigh sample.

Packaging Procedure:

- Double-wrap the sample with hexane-rinsed aluminum foil; fill out the sample tag and C-O-C form; tape the sample tag on outside of the sample; put C-O-C form into the envelope in the tray of the cooler in which samples are placed.

Recording Details:

- Record all general data.
- Record any unusual physical characteristics.
- Note weight, sex, and life stage.
- Describe the soil type.
- Identify the block number (mark location of trap grid on map).
- Identify the trap number where the mouse was collected (mark on grid map).

## A.2.4 STARLING

Collection:

Starling (*Sturnus vulgaris*)

Juveniles—On the back, a uniform dark olive-brown; below somewhat streaked with lighter markings at first, but soon become unicolor; white or buffy throat; after fall, they molt in synchrony with adults, cannot with certainty be distinguished from adults, although juveniles tend to have larger white tips to the feathers below.

Adults—Distinctive combination of black body and rather long, sharp, yellow bill; in the male, the base of the lower mandible is somewhat darkened with livid; in the female, these parts are simply paler yellow. After the fall molt in about mid-September, the feathers of the sides of the head, breast, flanks and underparts have white tips, so that from a distance the bird has a gray, mottled appearance. At close range, however, the dark parts of the feathers of the throat, breast, and flanks have iridescent reflections of purple, green, and blue; the back has green and bronze iridescence in brown-tipped feathers. During winter most of the white tips to the feathers on the breast and underparts wear off, leaving the bird dark below, with the iridescent reflections still present. About 8.5 inches long; weight about that of the robin, but the short drooping tail gives it, when at rest, a chunky, humpbacked appearance.

Verify adult association with nest box, if possible.

| Collection | | | | | |
|---|---|---|---|---|---|
| Timing | Locations and Numbers | Coordination/ Permits | Method | Age | Tissue |
| Nestlings– Summer, about 16 May to 21 June | Randomly selected nest boxes within each of the 5 group locations within the AOD | None | Obtain nestling from USFWS staff member within 1 hour of its removal from box by USFWS.<br><br>Put nestling specimen into clean cloth bag and place in asphyxiation cooler; remove from cloth bag before packaging; alternatively, use cervical separation. | Nestlings just prior to fledgling | Dressed carcasses composed of a whole bird without feathers, beak, keratinized leg parts, and GI tract; the removed tissues (except the GI tract) should be saved as a separate sample identified by the same sample tag number but with an R added |

(If specific approval is given by USFWS for nestlings to be collected by a SFS-Phase I staff member, use the following technique. Approach nest box gently, but so as to make adult aware; watch for adult to leave nest box; rapidly place ladder, remove nestling, close box and leave with minimum disturbance.)

- Prior to sample collection, coordinate with USFWS to avoid interfering with their ongoing starling monitoring program and to arrange the time for nestling transfer.

- Starling collection assumes that the USFWS starling monitoring program investigator will tell field team members when nestlings are within 3 days of fledgling, after which time the USFWS staff member will collect the nestling within 1 to 2 days of fledgling and turn it over alive to the field team member within 1 hour of collection so that the field team member can dispatch, handle, and prepare the sample according to SFS-Phase I protocols.

- If there are fewer than 50 starlings available when collecting an equal number from each of the 5 group locations within the AOD, collect more individuals from one of the 3 group locations that are close to or marginally within the AOD, randomly picking which of the group locations to use and which of the additional active nests to sample.

- If active starling nests are absent from a group location, check with the USFWS starling monitoring program investigator once every week to see if active nests have been established.

Sample Preparation:

- Follow detailed procedures for performing a necropsy on a starling provided in the SAP; following necropsy, the sample is comprised of a dressed carcass, which is defined as a whole starling minus the feathers, bill, tarsi, and gastrointestinal tract.

- Weigh sample.

Packaging Procedure:

- Double-wrap a sample with hexane-rinsed aluminum foil; fill out the sample tag and C-O-C form; tape the sample tag on the outside of the sample; put C-O-C form into the envelope in the tray of the cooler in which samples are placed.

- Save removed body parts (except gastrointestinal tract) preparing them as a residual sample, i.e., appending an "R" to the original sample number (e.g., B0458R). (Since only 10 percent of the residual samples are to be analyzed, i.e., five specimens within the AOD, randomly select the blocks from which residual samples are to be saved before collecting the first starling.)

- Weigh samples.

Recording Details:

- Record general data.
- Record any unusual physical characteristics.
- Identify group nest location.
- Identify nest box number.

## A.2.5 GROUND-DWELLING BEETLE

Collection:

| Ground-dwelling Beetle (Coleoptera)<br>Collect all species of beetles that end up in the pit trap. These have a horny or leathery elytra and a head that is narrower than the rest of the body. | | | | | |
|---|---|---|---|---|---|
| Collection | | | | | |
| Timing | Locations and Numbers | Coordination/ Permits | Method | Age | Tissue |
| Summer | Random locations within each of 25 blocks | None | Pit trap | All adults | Whole-body composite weighing a minimum of 20 g |

- Embed new and shiny paint cans (which have slippery sides) flush with the ground surface. When in use, check the traps every 1 to 3 days unless it rains, in which case check them daily. When traps are not in use, put lids on the cans.
- Check pit traps for 3 consecutive days to determine an appropriate checking frequency that can be used thereafter.
- If after 2 weeks of checking a given trap location, a sufficient mass of beetles has still not been collected, begin collecting a supplementary sample (to composite with the first sample) from a location 200 ft from the first location within the block along a randomly selected compass line.
- Composite enough ground-dwelling beetles from a small geographic area within each assigned sampling location to provide 20 g of sample.

Sample Preparation:

- Weigh specimens in jar by subtracting the tare from the gross weight.

Packaging Procedure:

- Place a composite sample in glass bottle. If the full sample weight is not obtained on the first day of sampling, the sample should be considered incipient and given an "F" sample number. Continue adding mass from repeated checking of the trap until the sample weighs at least 20 g.

- If sample weight allows, save at least one voucher specimen of at least the major types of ground-dwelling beetle collected in an ethanol-filled container.

- Once an adequate number of specimens has been collected, identify and count the numbers of individuals representing each reasonably identifiable taxon in the sample; record this information in the Laboratory Notebook and its attachments, as appropriate.

- Weigh specimens in bottle by subtracting the tare from the gross weight.

- After sample taxa have been identified and weighed, fill out the sample tag and C-O-C form; tape the sample tag on the outside of the bottle and put the lid on; put C-O-C form into the envelope in the tray of the cooler in which samples are placed.

Recording Details:

- Record all general data.

- Record any unusual physical characteristics.

- Describe the soil type.

- If edging is used to funnel beetles toward trap, record the dimensions and sketch on map the area where the funnel was used to obtain sample.

- Note number of apparent taxa in sample.

- Note number of individuals in sample.

- Identify or describe any other type of animal caught in pit trap (e.g., shrew, deer mouse).

- Note reasonably identifiable taxa in sample.

- In notes section, describe identified taxa (e.g., color, patterns) so that basis for identification is apparent.

## A.2.6 GRASSHOPPER

Collection:

Grasshopper (Acrididae)

Collect both species of short-horned and long-horned grasshoppers. Short-horned grasshoppers have short antennae and a broad, round face perpendicular to body. Long-horned grasshoppers have long antennae and a face that is often triangular and slanted with respect to the body axis.

| Collection | | | | | |
|---|---|---|---|---|---|
| Timing | Locations and Numbers | Coordination/ Permits | Method | Age | Tissue |
| Late Summer/ Early Fall | The same random locations within each of 25 blocks used to collect beetles | None | Sweep net along transects within a 100-ft (30-m) radius circle centered at the assigned sample location. Allow 3.3 ft (10 m) beyond location boundary for additional collection, if necessary. | Late instar nymphs (large, non-winged specimens), if possible | Whole-body composite weighing a minimum of 20 g |

- Composite enough grasshoppers from a small geographic area within each assigned sampling location to provide 20 g of sample.

- If after 5, generally consecutive days of sweeping at a given sampling location, a sufficient mass of grasshoppers has still not been collected from within a 100-ft radius, expand the radius of the circle being swept to 200 ft.

Sample Preparation:

- Weigh specimens in bottle by subtracting the tare from the gross weight.

- As part of the packaging procedure only, remove the hind legs (if they are well hardened and spiny) so they will be present during taxonomic identification.

Packaging Procedure:

- Place a composite sample in glass bottle. If the sample weight is not obtained on the first day of sampling, the sample should be considered incipient and given an "I" number. Continue adding mass from repeated sweeping of the area until incipient sample weighs at least 20 grams.

- If sample weight allows, save at least one voucher specimen of at least the major types of grasshoppers collected in an ethanol-filled container.

- Once an adequate number of specimens has been collected, identify and count the numbers of individuals representing each reasonably identifiable taxon in the sample; record this information in the Laboratory Notebook and its attachments, as appropriate.

- Remove hind legs before packaging if they are well hardened and spiny.

- Weigh sample in bottle by subtracting the tare from the gross weight.

- After sample taxa have been identified and weighed, fill out the sample tag and C-O-C form; tape the sample tag on the outside of the jar and put the lid on; put the C-O-C form into the envelope in the tray of the cooler in which samples are placed.

- Dispose of hind legs in garbage can.

Recording Details:

- Record all general data.

- Record any unusual physical characteristics.

- Describe the soil type.

- Indicate the dimensions of the sweep area by sketching them directly on the map and noting them in the Field Notebook.

- Note the number of taxa in sample.

- Note the number of individuals in sample.

- Note reasonably identifiable taxa in sample.

- In notes section, describe identified taxa (e.g., color, patterns) so that basis for identification is apparent.

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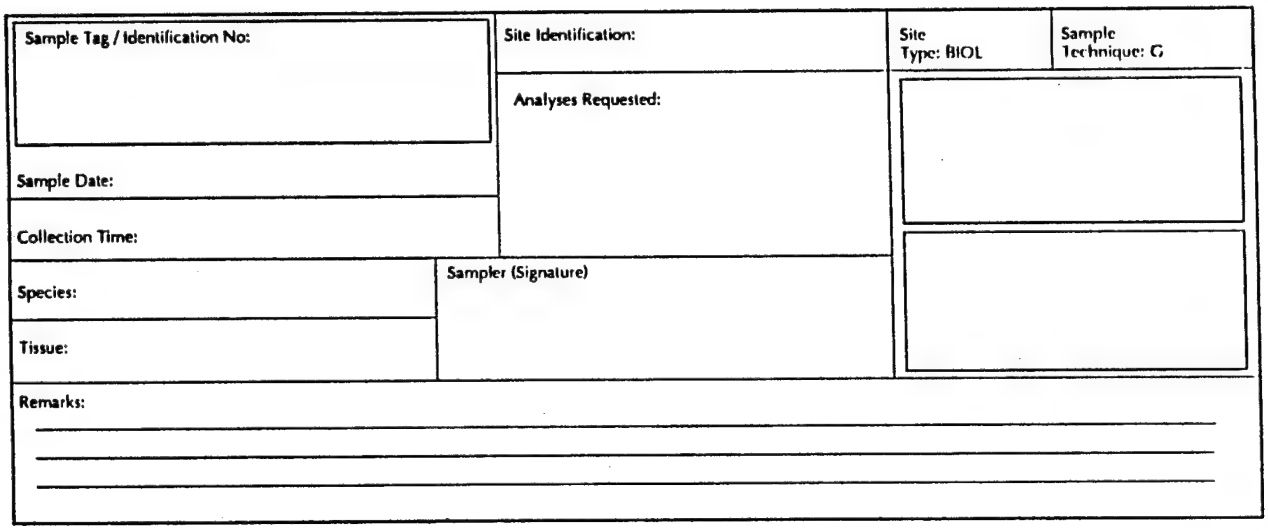


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**Figure A-1**

**Sample Tag**

Rocky Mountain Arsenal
Prepared by: Ebasco Services Incorporated

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**Figure A-2**

**Chain of Custody**

Rocky Mountain Arsenal
Prepared by: Ebasco Services Incorporated

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# Figure A-3

## Field Data Form

Rocky Mountain Arsenal
Prepared by: Ebasco Services Incorporated

| | SAMPLE TAG/ IDENTIFICATION NUMBER | SITE / AREA IDENTIFICATION | SAMPLE LOCATION: COUNTY | RANGE | TOWN-SHIP | SEC. | QTR. | NORTHING COORDINATES | EASTING COORDINATES | COLLECTION DATE: MO. DAY YR. | COLLECTION TIME (Military) | SAMPLE WEIGHT (gm) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | | | | | | | | | | | | |
| B | | | | | | | | | | | | |
| C | | | | | | | | | | | | |
| D | | | | | | | | | | | | |
| E | | | | | | | | | | | | |

Samplers:

Signature

Logbook and Page Number

Photo Log Number

| SPECIES | LIFE STAGE | SEX | TISSUE | COLLECTION METHOD | HABITAT TYPE |
|---|---|---|---|---|---|
| ACRI = Grasshopper<br>ANPL = Mallard<br>ATCU = Burrowing Owl<br>BRTE = Cheatgrass<br>BUVI = Great Horned Owl<br>CEDE = Coontail<br>CHVO = Killdeer<br>COLE = Ground Beetle<br>CYLU = Prairie Dog<br>ESLU = Northern Pike<br>FASP = American Kestrel<br>FUAM = Coot<br>HEAN = Sunflower<br>ICME = Black Bullhead<br>ICNE = Brown Bullhead<br>ICPU = Channel Catfish<br>KOIR = Kochia<br>LASE = Prickly Lettuce<br>LEMA = Bluegill<br>MISA = Bass<br>ODHE = Mule Deer<br>ODVI = Whitetail Deer<br>OLIG = Earthworm<br>PEMA = Deer Mouse<br>PHCO = Pheasant<br>PLAN = Plankton<br>POND = American Pondweed<br>POPE = Sego Pondweed<br>STNE = Meadowlark<br>SYAU = Desert Cottontail<br>WCIV = Water Column Macro Invertebrates<br>ZEMA = Mourning Dove<br>Other _____ | A = Adult/Mature<br>B = Juvenile<br>C = Egg<br>D = Seed Stage<br>E = Flowering stage<br>F = Rapid growth<br>G = All life stages | M = Male<br>F = Female<br>O = Not applicable<br>U = Unknown | 1 = Whole body<br>2 = Dressed carcass<br>3 = Egg<br>4 = Muscle tissue<br>5 = Liver tissue<br>6 = Brain tissue<br>7 = Composite<br>8 = Above substrate plant<br>9 = Leaves and flowering heads<br>10 = Heart<br>11 = Kidney<br>12 = Body fat<br>13 = Solid stomach contents<br>14 = Liquid stomach contents<br>15 = Other | 1 = Shotgun / steel shot<br>2 = Shotgun / rifle slug<br>3 = .22 Rifle<br>4 = Nest box<br>5 = Live trap<br>6 = Sweep net<br>7 = Dip net<br>8 = Gill net<br>9 = Trap net<br>10 = Beach seine<br>11 = Plankton net<br>12 = Nest capture<br>13 = Rake<br>14 = Scissors<br>15 = Shovel<br>16 = Fortuitous live<br>17 = Fortuitous dead | 1 = Annual Forb<br>2 = Cheatgrass with weedy forbe<br>3 = Cheatgrass with perennial grasses<br>4 = Perennial native grasses (heavy soil)<br>5 = Perennial native grasses (light soil)<br>6 = Crested wheatgrass<br>7 = Lacustrine<br>8 = Persistent emergent wetland<br>9 = Scrub-shrub wetland<br>10 = Riparian forest<br>11 = Riparian shrub<br>12 = Rabbitbrush<br>13 = Sand sagebrush<br>14 = Subshrubs & succulents<br>15 = Locust thickets<br>16 = Perennial grasses<br>17 = Ornamental trees & shrubs<br>18 = Cultivated<br>19 = Building area/pavement |

*SPECIFIC DATA*

| | NEST BOX/ TRAP ID NUMBER(S) | NUMBER OF INDIVIDUALS IN COMPOSITE | LENGTH (mm) | AGING TISSUE COLL'D | SOIL MAP UNIT | COLLECTION DEPTH (m) | NO. OF TAXA IN SAMPLE | VOLUME OF SOIL ($m^3$) | COLLECTION AREA ($m^2$) | EGGS: VOLUME (ml) | EGGS: WT. (gm) | EGGS: LN. (mm) | EGGS: WD. (mm) | SAMPLE VOLUME (ml) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Great Horned Owl<br>Deer Mice<br>Kestrels | Mice, Grasshoppers, Earthworms | Fish | Deer<br>Fish | Terr. Plants, Deer Mice, Earthworms, Prairie Dogs | Aquatic Plants, Earthworms | Grass-hoppers | Earthworms | Grasshoppers | Great Horned Owl<br>Kestrel | Great Horned Owl<br>Kestrel | G.H.Owl<br>Kestrel | G.H.Owl<br>Kestrel | Plankton |
| A | | | | | A = Antler | OBSERVATIONS AND ABNORMALITIES | | | | | | | | |
| B | | | | | F = Fin ray | | | | | | | | | |
| C | | | | | J = Jaw | | | | | | | | | |
| D | | | | | O = Otolith | | | | | | | | | |
| E | | | | | S = Scale | | | | | | | | | |

Rev. 3/5/90

# APPENDIX B

# FURTHER STATISTICAL DISCUSSIONS AND EVALUATIONS

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Table

# INTRODUCTION TO APPENDIX B

## FURTHER STATISTICAL DISCUSSIONS AND EVALUATIONS

Appendix B provides additional details to augment the information presented in the study design pertaining to the following topics: evaluation of the hypotheses for predators, selection of sample pattern, and the analysis of sample sizes.

Section B.1 describes the proposed data analysis for the predator (owl, kestrel, and eagle) evaluations. Section B.2 describes the rationale for selecting a random block sampling approach over a stratified sampling approach. Section B.3 provides more detail on the analysis of power and sample size for the prey and predator evaluations and includes five spreadsheets (Tables B.1 through B.5) which evaluate power and sample size under different assumptions regarding the underlying processes.

## B.1.0 DATA ANALYSIS FOR THE PREDATOR EVALUATIONS

### B.1.1 BALD EAGLE

The equation for estimating the mean terrestrial HQ for the eagle is given in Table B.3. The mean HQ is based on the mean dose which is calculated from the food web model equation and the mean prairie dog tissue concentration within the eagle exposure area. Table B.3 also provides formulas for calculating the upper confidence interval for the mean. These formulas are useful in assessing the adequacy of the prairie dog sample size even though the confidence interval is not incorporated into the decision boxes for eagles.

The mean prairie dog tissue concentration $\overline{TC}_{pdog}$ is estimated from the means derived from the AOD and bald eagle exposure area outside the AOD as follows.

$$TC_{pdog} = W_1 * TC_{pdog,1} + W_2 * TC_{pdog,2}$$

$$W_1 = \frac{\text{Area of 1993 Prairie Dog Towns in AOD}}{\text{Area of All Prairie Dog Towns}}$$

$$W_2 = 1 - W_1$$

The variance of the mean, which is used to calculate confidence intervals and assess sample size adequacy, is given by:

$$V(TC_{pdog}) = \tilde{W_1} * V(TC_{pdog,1}) + \tilde{W_2} * V(TC_{pdog,2})$$

$$V(TC_{pdog,k}) = V(TC_{pdog,k}) / N_{pdog,k}$$

where $\overline{TC}_{pdog1}$ and $\overline{TC}_{pdog2}$ are the means estimated from samples taken from the AOD and bald

eagle exposure area outside the AOD (excluding portions in the prey study are), respectively. These formulas ensure that equal areas of the bald eagle study area receive equal weight in deriving the overall mean.

### B.1.2 OWL AND KESTREL

The evaluations for predators (other than the eagle) are based on the upper confidence limit estimate of the mean and a point estimate of the 90th percentile. The estimation of the mean HQ for the predator is relatively straightforward because the average dose is proportional to the average prey tissue concentrations within the AOD. The reasoning behind this is as follows. The interest focuses on calculating the mean dose received by a hypothetical population of predators that feeds entirely within the AOD. These predators each receive a dose proportional to the mean prey tissue concentration within a given exposure zone. To calculate the mean predator dose, the mean prey tissue concentration can be calculated for a representative sample of all possible exposure zones, and then these exposure zone means are averaged to produce a single overall mean. However, the mean of the exposure zone means is equal to the mean of all prey tissue concentrations within the AOD. Therefore, the size of the predators exposure zone is irrelevant in calculating the average dose for individuals feeding within the AOD. Likewise the variance of the mean dose, and therefore the confidence intervals, can be calculated based on the variance in the mean prey tissue concentrations in the AOD. These formulas are shown in Tables B.4 and B.5.

The estimation of HQ90 is less straightforward than the estimation of the mean because it depends on the variance of the exposure zone mean tissue concentrations, which in turn depends on the size of the predator exposure zone. Intuitively, larger exposure zones imply lower variances in dose and, therefore, lower 90th percentile doses. The estimation of HQ90 requires a spatial interpolation of prey tissue concentrations and subsequent averaging within a representative subset of predator exposure zones. The kestrel, and particularly the owl, exposure zones are too large to fit entirely within the AOD. Therefore, the meaning and interpretation of the 90th percentile dose is ambiguous. (The interpretation of the mean dose is somewhat less

ambiguous because the mean does not theoretically depend on the exposure zone size.) However, because RMA Council guidance requires the estimation of a HQ90, the following method was recommended. The AOD will be divided into 10 irregularly shaped zones of approximately equal area and the mean prey tissue concentration within each of these areas will be calculated. The 10 values for HQ will be estimated from the 10 mean prey tissue concentrations calculated for these zones and the 9th largest value will be used for HQ90. This method will effectively estimate the 90th percentile HQ for hypothetical predators with the same feeding rates but smaller exposure ranges than the owl and kestrel. Because this area is smaller than that of the owl and kestrel exposure ranges, the estimation of HQ90 will be biased upward to some extent. The recommended approach maximizes the variance of the HQ estimates, and thus maximizes HQ90. If larger or overlapping zones are specified, the variance in the resulting HQs will decrease.

B.1.3.1 Additional Notes on Derivation of Formulas for Predators

Because sample size estimation is only approximate and based on assumptions, it was not worthwhile to complicate the analysis by including species that contributed only minute fractions (<0.03) to a predator's diet. Fractions for the remaining individuals were readjusted. For owls and eagles, the dietary fraction for medium mammals will be associated entirely with cottontails and prairie dogs, respectively, since these species comprise the main dietary fraction for the respective predator.

B.1.3.2 Correlation Term in the Predator Variance Equations

The variance of the mean predator dose depends on all of the pairwise correlations among the mean tissue concentrations for the prey items. The true correlation between the means may well be high. It will be higher than the correlation between individual samples taken at a given location or between an individual sample and soil concentration. Because higher correlations imply higher variances and higher sample sizes, a maximal correlation of 1.0 was assumed. During the SFS-Phase 1 data analysis, alternate correlations can be incorporated, although they would result in smaller (less conservative) confidence intervals.

## B.2.0 RATIONALE FOR SELECTING A RANDOM BLOCK SAMPLING APPROACH OVER A STRATIFIED SAMPLING APPROACH

In cases where the underlying population exhibits trends or distinct patterns over space, it is sometimes efficient to divide the population into strata and allocate samples to each stratum based on its expected variance. The global mean, percentiles, and confidence intervals are then calculated based on the sample mean and variance within each stratum. If the underlying populations do not exhibit distinct strata or the strata boundaries and variances are not well known, the stratified approach may be less efficient than random sampling and produce biased estimates. A stratified sampling design within the prey or predator AOD would likely be very complex, especially if it were to consider multiple chemicals and species. Because current data provide little information on variances in different locations across the AOD, the study design was not based on stratified sampling methodology.

## B.3.0 ANALYSIS OF POWER AND SAMPLE SIZE

### B.3.1 DEFINITION OF POWER

Power is defined as the probability of rejecting the null hypothesis when it is in fact false and should be rejected. Power is calculated for a specific "detectable difference" between the true mean and the action level of 1.0. For example, the probability of rejecting the null hypothesis ("meanHQ > 1") is higher when the true mean is 0.3 than when the true mean is 0.95 for a given sample size. Conversely, the detectable difference can be calculated for a given level of power. In the following analysis, the detectable difference is calculated for a power of 0.8. Power also depends on the variances of the underlying population. For predators, power is dependent on the prey means as well as variances.

### B.3.2 GENERAL BACKGROUND FOR CALCULATIONS USED IN SPREADSHEETS

Table B.1 describes the effect of sample size and variance on power in evaluating the prey meanHQ. Results for two types of tests are given: one for low skewness (t-based, Gilbert 1987) and one for high skewness (H-based, Gilbert 1987). None of the calculations reflect adjustment for the presence of BCRLs. Depending on the fraction of BCRLs in the data and the closeness

of the CRL to the MATC, the calculations will under represent the true power for a given sample size (n). The following variables, which reflect statistical power, are calculated based on n and an assumed population standard deviation (SD) as follows:

- The maximum detectable mean: the highest value for the true mean that can be differentiated from 1.0 with a power of 0.8 (i.e., maximum detectable mean equals 1.0 minus detectable difference). Power formulas (Zar 1984) were readily available only for the t-based test.

- The error term (E): the term that must be added to or multiplied by the sample mean, depending on the type of test, to obtain the UCL.

- The critical value (CV) for the sample mean: the highest value of the sample mean that will result in a rejection of the null hypothesis, given the assumed variance. Negative values arise for the low skewness test when the data cannot possibly meet the assumption of normality and at the same time have a mean < 1.0. This variable gives only a rough indication of power. It is included for the purpose of comparing the low- and high-skewness tests.

- Terms required for calculation of E and CV for high skewness: log variance and H statistic. In the H-based equations, CV must be determined by iteration. Different values of CV imply different log variances (degrees of skewness) that in turn imply different H statistics and CVs. The CVs finally chosen imply an UCL of approximately 1.00. Formulas for power were not readily available; however, the CVs provide an informal indication of power.

The calculations for low skewness can be used if the sample size is high relative to the skewness of the data. Obvious cases where this condition is not met can be seen in Table B.1, where the CVmean for the test for low skewness becomes negative. Under high skewness, the high skewness test is the most valid, even though it has wider confidence intervals (lower power), and therefore sample sizes should be chosen accordingly.

Table B.2 shows the effect of sample size on the estimation of the UCL on HQ90. Although the objectives state that the 90th percentile itself and not the UCL is to be estimated, the UCL allows the accuracy in estimating the 90th percentile to be evaluated for a given sample size. The UCL is evaluated based on a parametric test for a normally distributed population (Gilbert 1987) and a nonparametric test for cases where the distribution is unknown (Gilbert 1987). For the

nonparametric test, the HQ90 and its UCL are calculated based on the order statistics of the data set. Therefore the CV for the mean or for HQ90 cannot be estimated. Formulas for calculating power for these two tests were not readily available. To provide insight on the relative power from different sample sizes, the following variables are given in Table B.2.

- The error/variance term: the term added to the sample mean to provide a parametric estimate of HQ90 and UCL for HQ90.

- The confidence interval: the difference between the UCL and the unbiased estimate of HQ90.

- The critical value for the sample mean: the highest value of the sample mean that will result in a rejection of the null hypothesis that HQ90 > 1 based on the parametric test and a 95 percent confidence level. Negative values indicate that the data cannot possible meet the assumption of normality and at the same time have a UCL HQ90 < 1.

- The order statistic (OS) used in the nonparametric estimate for the HQ90. For example, the 96th highest value out of 100 samples provides the approximate UCL on the 90th percentile. (Actual calculation of the UCL would involve linear interpolation rather than rounding up.)

- The approximate percentile represented by the order statistic used for the UCL (i.e., OS/N * 100).

Tables B.3, B.4, and B.5 show the effect of prey sample sizes, means, and variances on power for the mean HQ evaluations for the eagle, owl, and kestrel, respectively. The formulas for these tables were derived based on the dose model formula and standard statistical theory relating to the variances of products and sums. The formulas are given in the tables. The maximum detectable mean is specified based on the low power skewness formula (Zar 1984). The assumption of low skewness is reasonable because the predator means depend on the mean TCs for prey. The uncertainty distributions for mean prey TC are likely to have low skewness even when prey TCs are themselves skewed.

## B.4.0 REFERENCES CITED

Gilbert, R.O.
1987. Statistical Methods for Environmental Pollution Monitoring. Van Nostrand Reinhold.

Zar, G.
1984. Biostatistical Analysis. Prentice-Hall, Inc.

Table B-1 Sample Size and Power for Testing the Mean Hazard Quotient for Prey Species



| | | Low Skewness[1/] | | |
|---|---|---|---|---|
| | | max detectable mean at power = 0.8 | UCL = mean * E | |
| N | SD | max detectable mean | E | CV mean |
| 30 | 0.1 | 0.9536444142 | 0.0310 | 0.97 |
| 50 | 0.1 | 0.9644042446 | 0.0237 | 0.98 |
| 100 | 0.1 | 0.9750000000 | 0.0166 | 0.98 |
| 200 | 0.1 | 0.9823718279 | 0.0116 | 0.99 |
| 30 | 0.50 | 0.7682220711 | 0.1551 | 0.84 |
| 50 | 0.75 | 0.7330318348 | 0.1779 | 0.82 |
| 100 | 0.75 | 0.8125000000 | 0.1246 | 0.88 |
| 200 | 0.50 | 0.9118591397 | 0.0582 | 0.94 |
| 30 | 1.00 | 0.5364441422 | 0.3102 | 0.69 |
| 50 | 1.00 | 0.6440424464 | 0.2372 | 0.76 |
| 100 | 1.00 | 0.7500000000 | 0.1661 | 0.83 |
| 200 | 1.00 | 0.8237182795 | 0.1163 | 0.88 |
| 30 | 2.00 | 0.728882843 | 0.6204 | 0.38 |
| 50 | 2.00 | 0.2880848927 | 0.4743 | 0.53 |
| 100 | 2.00 | 0.5000000000 | 0.3322 | 0.67 |
| 200 | 2.00 | 0.6474365589 | 0.2326 | 0.77 |

1/ Low Skewness

HQ = hazard quotient
N = sample size
SD = standard deviation of HQ
power = 0.8
max detectable mean = maximum true mean HQ that can be differentiated from 1.0 with a probability of at least 0.8 (power of 0.8)
= 1 - (SD/sqrt(N)) * (Z(0.05) + Z(0.2))
UCLmean = upper confidence limit on the mean HQ
= mean + E
E = error term
= t(0.05, N-1) * SD/sqrt(N)
CVmean = critical value for mean HQ
= value of sample mean that would produce an UCL = 1.0
= 1 - E
Z = tabled value from a standardized normal distribution
t = tabled value from a t distribution
sqrt = square root

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Table B-1 Sample Size and Power for Testing the Mean Hazard Quotient for Prey Species



| | High Skewness[2/] | | | | |
|---|---|---|---|---|---|
| | | | | UCL = mean * E | |
| N | CoefVar | lnSD | H(1-.05) | E | CV mean |
| 30 | 0.533 | 0.500 | 1.928 | 1.3553 | 0.74 |
| 50 | 0.533 | 0.500 | 1.876 | 1.2956 | 0.77 |
| 100 | 0.533 | 0.500 | 1.830 | 1.2423 | 0.80 |
| 200 | 0.533 | 0.500 | 1.830 | 1.2091 | 0.83 |
| 30 | 1.311 | 1.000 | 2.423 | 2.5856 | 0.39 |
| 50 | 1.311 | 1.000 | 2.306 | 2.2920 | 0.44 |
| 100 | 1.611 | 1.000 | 2.205 | 2.0577 | 0.49 |
| 200 | 1.311 | 1.000 | 2.205 | 1.9277 | 0.52 |
| 30 | 2.913 | 1.500 | 3.077 | 7.2578 | 0.14 |
| 50 | 2.913 | 1.500 | 2.881 | 5.7108 | 0.18 |
| 100 | 2.913 | 1.500 | 2.713 | 4.6367 | 0.22 |
| 200 | 2.913 | 1.500 | 2.8713 | 4.1102 | 0.24 |
| 30 | 7.321 | 2.000 | 3.812 | 30.4395 | 0.03 |
| 50 | 7.321 | 2.000 | 3.533 | 20.2758 | 0.05 |
| 100 | 7.321 | 2.000 | 3.295 | 14.3295 | 0.07 |
| 200 | 7.321 | 2.000 | 3.295 | 11.7888 | 0.08 |

HQ = hazard quotient
N = sample size
lnmean = mean of natural log-transformed HQ
lnSD = standard deviation of natural log-transformed HQ
= sqrt(ln((CoefVar)^2 + 1))
CoefVar = coefficient of variation
= SD/mean
UCLmean = upper confidence limit on the mean HQ
= exp(lnmean + 0.5 * lnSD^2 + lnSD * H(0.95, lnSD)/sqrt(N-1))
= mean * E
E = error term
= exp(0.5 * lnSD^2 + lnSD * H(0.95, lnSD)/sqrt(N-1))
CVmean = critical value for mean
= value of mean that would produce an UCL = 1.0
= 1 / E
H = Tabled value from Gilbert (1987; Table A.10)

Table B-2 Effect of Sample Size in Estimating HQ90 and the 95% UCL for HQ90 for Prey Species



| | | PARAMETRIC | | | | NON-PARAMETRIC | |
|---|---|---|---|---|---|---|---|
| | | UCL = mean+E1 | UE = mean+E2 | CI = UCL-UE | | UCL = HQdata(OS) | UCL |
| N | SD | E1 | E2 | CI | CVmean | OS(interpolate) | Approximate Percentile |
| 30 | 0.1 | 0.1778 | 0.1282 | 0.0496 | 0.82 | 30.60 | 102 |
| 50 | 0.1 | 0.1646 | 0.1282 | 0.0364 | 0.84 | 49.39 | 99 |
| 100 | 0.1 | 0.1527 | 0.1282 | 0.0245 | 0.85 | 95.84 | 96 |
| 200 | 0.1 | 0.1481 | 0.1282 | 0.0199 | 0.85 | 187.88 | 94 |
| 30 | 0.5 | 0.8890 | 0.6410 | 0.2480 | 0.11 | 30.60 | 102 |
| 50 | 0.5 | 0.8230 | 0.6410 | 0.1820 | 0.18 | 49.39 | 99 |
| 100 | 0.5 | 0.7635 | 0.6410 | 0.1225 | 0.24 | 95.84 | 96 |
| 200 | 0.5 | 0.7405 | 0.6410 | 0.0995 | 0.26 | 187.88 | 94 |
| 30 | 1.0 | 1.7780 | -0.7800 | 2.5560 | NC | 30.60 | 102 |
| 50 | 1.0 | 1.6460 | -0.6500 | 2.2920 | NC | 49.39 | 99 |
| 100 | 1.0 | 1.5270 | -0.5300 | 2.0540 | NC | 95.84 | 96 |
| 200 | 1.0 | 1.4810 | -0.4800 | 1.9620 | NC | 187.88 | 94 |
| 30 | 2.0 | 3.5560 | -2.5600 | 6.1120 | NC | 30.60 | 102 |
| 50 | 2.0 | 3.2920 | -2.2900 | 5.5840 | NC | 49.39 | 99 |
| 100 | 2.0 | 3.0540 | -2.0500 | 5.1080 | NC | 95.84 | 96 |
| 200 | 2.0 | 2.9620 | -1.9600 | 4.9240 | NC | 187.88 | 94 |

Approximate Percentile of data to get UCL = 100 * OS/N
Approximate Percentile of data to get UE = 90th
HQ = hazard quotient
SD = standard deviation of HQ
HQ90 = 90th percentile HQ
= order statistic of the HQ data
UE = unbiased estimate of HQ90
= mean + E2
UCL = upper confidence limit on HQ90
= mean + E1
CI = one-sided confidence interval on HQ90
= UCL - UE

CVmean = critical value
= value of sample mean that would result in UCL = 1.0
= 1 - E1
E1 = SD * K(0.95, p=0.9)
E2 = SD * Z(0.1)
Z = tabled value from a standard normal distribution
K = tabled value from Gilbert, 1987, Table A3
OS = order statistic of data that provides an estimate of UCL
= 0.9 * (N+1) + Z(0.05)*sqrt(N*0.9*(1-0.9))

Table B-3 Calculation of Power for Bald Eagle

| CASE 1 | |
|---|---|
| SD(HQpdog) = | 0.1 |
| Npdog = | 50 |
| meanHQpdog = | 0.05 |
| MATC(pdog) = | 0.19 |
| meanTCpdog = | 0.0095 |
| meanR = | 0.089 |
| V(meanR) = | 0.0007232 |
| V(meanTCpdog) = | 0.00000722 |
| | |
| meanDOSE = | 0.0008455 |
| V(meanDOSE) = | 1.2768E-07 |
| meanHQ = | 0.42275 |
| V(meanHQ) = | 0.031919981 |
| | |
| power level = | 0.8 |
| Z(1 - power level) = | 0.84 |
| Max Detectable Mean = | 0.556025829 |

| CASE 2 | |
|---|---|
| SD(HQpdog) = | 0.2 |
| Npdog = | 1000 |
| manHQpdog = | 0.1 |
| MATCpdog = | 0.19 |
| meanR = | 0.089 |
| meanTCpdog = | 0.019 |
| V(meanR) = | 0.0007232 |
| V(meanTCpdog) = | 0.000001444 |
| | |
| meanDOSE = | 0.001691 |
| V(meanDOSE) = | 2.7355E-07 |
| meanHQ = | 0.8455 |
| V(meanHQ) = | 0.068389356 |
| | |
| power level = | 0.8 |
| Z(1 - power level) = | 0.84 |
| Max Detectable Mean = | 0.350138736 |

Quantities refer to bald eagle except where noted by "pdog"

| | | |
|---|---|---|
| HQ | = | hazard quotient for bald eagle |
| HQpdog | = | hazard quotient for prairie dog |
| Npdog | = | sample size for prairie dog |
| R | = | feeding rate for bald eagle |
| meanR | = | 0.089 |
| TCpdog | = | tissue concentration of prairie dogs |
| meanTCpdog | = | MATCpdog * meanHQpdog |
| V(meanTCpdog) | = | $MATC^2pdog * SD(HQpdog)^2 / Npdog$ |
| DOSE | = | dose to bald eagle |
| meanDOSE | = | meanR * meanTCpdog |
| V(meanDOSE) | = | $meanR^2 * V(meanTCpdog) + meanTCpdog^2 * V(meanR) + V(meanR) * V(meanTCpdog)$ |
| meanHQ | = | meanDOSE/TRV |
| V(meanHQ) | = | $V(meanDOSE)/TRV^2$ |
| UCL meanHQ | = | meanHQ + sqrt(V(meanHQ))*Z(.05) |
| max detectable mean | = | maximum true mean HQ which can be differentiated from 1.0 with probability of at least 0.8 |
| SD | = | standard deviation |
| MATCpdog | = | maximum allowable tissue concentration for prairie dog |
| TRV | = | toxicity reference value = 0.002 |
| Z | = | tabled value from standard normal distribution |

## Table B-4 Calculation of Power for Great Horned Owl

| CASE 1 | | | |
|---|---|---|---|
| meanHQpdog = | 0.1 | | |
| meanHQcot = | 0.1 | | |
| SD(HQ)pdog = | 0.2 | | |
| SD(HQ)cot = | 0.2 | | |
| Npdog = | 50 | | |
| Ncot = | 50 | | |
| meanHQmm = | 0.1 | meanHQsm = | 0.1 |
| SD(meanHQ)mm = | 0.028284271 | SD(HQ)sm = | 0.2 |
| | | N = | 50 |
| MATCmm = | 0.19 | MATCsm = | 0.19 |
| meanTCmm = | 0.019 | meanTCsm = | 0.019 |
| V(meanTCmm) = | 0.00002888 | V(meanTCsm) = | 0.00002888 |
| meanR = | 0.089 | | |
| V(meanR) = | 0.0007232 | | |
| mean(SUM) = | 0.019 | | |
| V(SUM) = | 0.00002888 | | |
| meanDOSE = | 0.001691 | | |
| V(meanDOSE) = | 5.1072E-07 | | |
| meanHQ = | 0.42275 | | |
| V(meanHQ) = | 0.031919981 | | |
| power level = | 0.08 | | |
| Z(1 - power level) = | 0.84 | | |
| max detectable mean | 0.556025829 | | |

| CASE 2 | | | |
|---|---|---|---|
| meanHQpdog = | 0.2 | | |
| meanHQcot = | 0.2 | | |
| SD(HQ)pdog = | 0.4 | | |
| SD(HQ)cot = | 0.4 | | |
| Npdog = | 1000 | | |
| Ncot = | 1000 | | |
| meanHQmm = | 0.2 | meanHQsm = | 0.2 |
| SD(meanHQ)mm = | 0.12649111 | SD(HQ)sm = | 0.4 |
| | | N = | 1000 |
| MATCmm = | 0.19 | MATCsm = | 0.19 |
| meanTCmm = | 0.038 | meanTCsm = | 0.038 |
| V(meanTCmm) = | 0.000005776 | V(meanTCsm) = | 0.000005776 |
| meanR = | 0.089 | | |
| V(meanR) = | 0.0007232 | | |
| mean(SUM) = | 0.038 | | |
| V(SUM) = | 0.000005776 | | |
| meanDOSE = | 0.003382 | | |
| V(meanDOSE) = | 1.09423E-06 | | |
| meanHQ = | 0.8455 | | |
| V(meanHQ) = | 0.068389356 | | |
| power level = | 0.8 | | |
| Z(1 - power level) = | 0.84 | | |
| max detectable mean | 0.350138736 | | |

Quantities refer to great horned owl except where noted for prey species

| | | |
|---|---|---|
| HQ | = | hazard quotient for great horned owl |
| HQprey | = | hazard quotient for prey |
| Nprey | = | sample size for prey |
| R | = | feeding rate for great horned owl |
| meanR | = | 0.089 |
| TCprey | = | tissue concentration of prey |
| meanTCprey | = | MATCprey * meanHQprey |
| meanHQmm | = | 0.0 * meanHQpdog + 1.0 * meanHQcot |
| V(meanTCmm) | = | 0.0^2 * V(meanHQpdog) + 1.0^2 * V(meanHQcot) |
| | | + 2 * 0.0 * 1.0 * SQRT(VmeanHQpdog) * V(meanHQcot)) * RHOpdog_cot |
| DOSE | = | dose to great horned owl |
| meanDOSE | = | meanR * SUM(meanTCj * FRj) |
| | | meanR * {meanTCmm * .87 + meanTCsm * .13} |
| V(meanDOSE) | = | meanR^2 * V(SUM) + mean(SUM)^2 * V(meanR) + V(meanR) * V(SUM) |
| V(SUM) | = | V(meanTCmm * .87) + V(meanTCsm * .13) |
| | | + 2 * .87 * .13 * SQRT(V(meanTCmm) * V(meanTCsm)) * RHOmm_sm |
| meanHQ | = | meanDOSE/TRV |
| V(meanHQ) | = | meanHQ + SQRT(V(meanHQ))*Z(0.05) |
| max detectable mean | = | maximum true mean which can be differentiated from 1.0 with probability of at least 0.8 |
| | = | 1 - (SQRT(V(meanHQowl) * (Z(confidence level) + Z(power level))) |
| max detectable mean | = | maximum true mean which can be differentiated from 1.0 with probability of at least 0.8 |
| RHO(all) | = | correlation = 1 |
| MATCprey | = | maximum allowable tissue concentration for prey = 0.19 |
| TRV | = | toxicity reference value for great horned owl = 0.004 |
| pdog | = | prairie dog |
| cot | = | cottontail |

Table B-5 Calculation of Power for American Kestrel

| CASE 1 | | | | | |
|---|---|---|---|---|---|
| SD(HQ)sm = | 0.3 | SD(HQ)sb = | 0.3 | SD(HQ)in = | NA |
| Nsm = | 50 | Nsb = | 50 | Nin = | 50 |
| meanHQsm = | 0.15 | meanHQsb = | 0.15 | meanHQin = | NA |
| MATCsm = | 0.19 | MATCsb = | 0.15 | MATCin = | NA |
| meanTCsm = | 0.0285 | meanTCsb = | 0.0225 | meanTCin = | 0.105 |
| V(meanTCsm) = | 0.00006498 | V(meanTCsb) = | 0.000405 | V(meanTCin) = | 0.0042 |
| meanR = | 0.089 | | | TC factor = | 0.15 |
| V(meanR) = | 0.0007232 | | | | |
| mean(SUM) | 0.042315 | | | | |
| COVterm(SUM) = | 0.000164279 | | | | |
| V(SUM) = | 0.000347419 | | | | |
| meanDOSE = | 0.003766035 | | | | |
| V(meanDOSE) = | 4.2809E-06 | | | | |
| meanHQ = | 0.3766035 | | | | |
| V(meanHQ) = | 0.42980935 | | | | |
| power level = | 0.8 | | | | |
| Z(1-power level) = | 0.84 | | | | |
| max detectable mean = | 0.48481368 | | | | |

| CASE 2 | | | | | |
|---|---|---|---|---|---|
| SD(HQ)sm = | 0.4 | SD(HQ)sb = | 0.4 | SD(HQ)in = | NA |
| Nsm = | 200 | Nsb = | 200 | Nin = | 200 |
| meanHQsm = | 0.2 | meanHQsb = | 0.2 | meanHQin = | NA |
| MATCsm = | 0.19 | MATCsb = | 0.15 | MATCin = | NA |
| meanTCsm = | 0.089 | meanTCsb = | 0.03 | meanTCin = | 0.14 |
| V(meanTCsm) = | 0.00002888 | V(meanTCsb) = | 0.000018 | V(meanTCin) = | 0.0014 |
| meanR = | 0.089 | | | TC factor = | 0.2 |
| V(meanR) = | 0.0007232 | | | | |
| mean(SUM) | 0.05642 | | | | |
| COVterm(SUM) = | 6.37369E-05 | | | | |
| V(SUM) = | 0.000128286 | | | | |
| meanDOSE = | 0.00502138 | | | | |
| V(meanDOSE) = | 3.41103E-06 | | | | |
| meanHQ = | 0.502138 | | | | |
| V(meanHQ) = | 0.034110306 | | | | |
| power level = | 0.8 | | | | |
| Z(1 - power level) = | 0.84 | | | | |
| max detectable mean = | 0.541045959 | | | | |

Quantities refer to kestrel except where noted for prey species

| | | |
|---|---|---|
| HQ | = | hazard quotient for kestrel |
| HQprey | = | hazard quotient for prey |
| Nprey | = | sample size for prey |
| R | = | feeding rate for kestrel |
| meanR | = | 0.089 |
| TCprey | = | tissue concentration of prey |
| meanTCprey | = | MATCprey * meanHQprey |
| DOSE | = | dose to kestrel |
| meanDOSE | = | meanR * SUM($meanTC_i$ * $FR_i$) |
| | = | meanR * ( meanTCsm * .69 + meanTCsb * .12 + meanTCin * .19) |
| V(meanDOSE) | = | $meanR^2$ * V(SUM) + $mean(SUM)^2$ * V(meanR) + V(meanR) * V(SUM) |
| V(SUM) | = | V(meanTCsm * .69) + V(meanTCsb * .12) + V(meanTCin * .19)<br>+ 2 * (.69* .12 *SQRT(V(meanTCsm) * V(meanTCsb)) * RHOsm_sb)<br>+ 2 * (.12* .19 *SQRT(V(meanTCsb) * V(meanTCin)) * RHOsb_in)<br>+ 2 * (.69* .19 *SQRT(V(meanTCsm) * V(meanTCin)) * RHOsm_in) |
| RHO(all) | = | 1 |
| meanHQ | = | meanDOSE/TRV |
| V(meanHQ) | = | V(meanDose)$TRV^2$ |
| UCL meanHQ | = | meanHQ + SQRT(V(meanHQ) * Z(0.05) |
| max detectable mean | = | maximum true mean which can be differentiated from 1.0 with probability of at least 0.8 |
| | = | 1 - (SQRT(V(meanHQkest) * (Z(confidence level) + Z(power level))) |
| MATCprey | = | maximum allowable tissue concentration = 0.19 |
| TRV | = | toxicity reference value for kestrel = 0.01 |

FOR INSECTS ASSUME:
meanTCin = mean TCdata * TC factor
V(meanTCin) = 2 * meanTCin/Nin
RHO(all) = Correlation = 1